The Conversion Gradient at *HIS4* of *Saccharomyces cerevisiae*. I. Heteroduplex Rejection and Restoration of Mendelian Segregation

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ABSTRACT

In *Saccharomyces cerevisiae*, some gene loci manifest gradients in the frequency of aberrant segregation in meiosis, with the high end of each gradient corresponding to a hotspot for DNA double-strand breaks (DSBs). The slope of a gradient is reduced when mismatch repair functions fail to act upon heteroduplex DNA—aberrant segregation frequencies at the low end of the gradient are higher in the absence of mismatch repair. Two models for the role of mismatch repair functions in the generation of meiotic "conversion gradients" have been proposed. The heteroduplex rejection model suggests that recognition of mismatches by mismatch repair enzymes limits hybrid DNA flanking the site of a DSB. The restoration-conversion model proposes that mismatch repair does not affect the length of hybrid DNA, but instead increasingly favors restoration of Mendelian segregation over full conversion with increasing distance from the DSB site. In our experiment designed to distinguish between these two models, data for one subset of well repairable mismatches in the *HIS4* gene failed to show restoration-type repair but did indicate reduction in the length of hybrid DNA, supporting the heteroduplex rejection model. However, another subset of data manifested restoration-type repair, indicating a relationship between Holliday junction resolution and mismatch repair. We also present evidence for the infrequent formation of symmetric hybrid DNA during meiotic DSB repair.

DURING prophase of meiosis, the budding yeast *Saccharomyces cerevisiae* introduces double-strand breaks (DSBs) in its DNA at discrete sites (hotspots; Sun *et al.* 1989; Nag and Petes 1993; Fan *et al.* 1995). The repair of these lesions is usually effected using the homologous chromosome (as opposed to the sister chromatid) as jig and template, and can result in crossing over. If the two homologs are genetically marked near the break (alleles *A* and *a*), DSB repair (DSBR) can result in aberrant segregation of the markers, which is a deviation from normal 2*A*:2*a* Mendelian segregation. The predominant types of aberrant segregation in yeast are full conversion (FC; 3*A*:1*a* and 3*a*:1*A*) and half conversion (HC). In HC events, one of the meiotic products has a sectored (*A*/*a*) genotype and is indicative of heteroduplex (mismatch-containing) DNA at the site of the marker. To describe events, we adopt a variation of the nomenclature of eight-spored fungi. HC tetrads, with one *A* spore, two *a* spores, and one spore that is *A*/*a*, are referred to as 5:3 tetrads, as are those with one *a*, two *A*, and one *A*/*a* spore. Tetrads with two *A* spores and two *a* spores (Mendelian segregation) are called 4:4 tetrads. FC tetrads are called 6:2 (*i.e.*, 6*A*:2*a* or 6*a*:2*A*). Aberrant 4:4 (ab4:4) tetrads, sometimes seen in yeast (and more frequently in other fungi), have one *A* spore, one *a* spore, and two spores that are *A*/*a* sectored (for review, see Petes *et al.* 1991). HC and ab4:4 tetrads are referred to collectively as postmeiotic segregation (PMS) tetrads.

One model for DSBR is presented in Figure 1. DSBR models for yeast meiosis (*e.g.*, Szostak *et al.* 1983; Gilbertson and Stahl 1996) agree on a number of basic features. The DNA strands ending 5′ at the break are resected through the action of an exonuclease. This exposes 3′-ending single-stranded tails, which invade a homologous DNA duplex in a reaction catalyzed by a RecA-like enzyme (*e.g.*, Rad51, Dmc1; reviewed in Kuzminov 1996). This invasion forms hybrid DNA (hDNA) between the two homologs. The 3′ ends of the invading molecule then prime DNA synthesis, using the invaded molecule as a template. This replaces the degraded segments with segments that have derived their sequence from the invaded molecule. After DNA synthesis, the two DNA molecules are held together in a "joint-molecule" structure in which two Holliday junctions (HJs) flank the region of DSBR (Collins and Newlon 1994; Schwacha and Kleckner 1994, 1995). To separate the joined molecules, this structure must be resolved. Some of the resolutions (~35% in *S. cerevisiae*) result in the swapping of arms (exchange) between the interacting molecules. Thus, DSBR can result in both gene conversion and crossing over.

Enzymatic repair of mismatches (MMR) in hetero-

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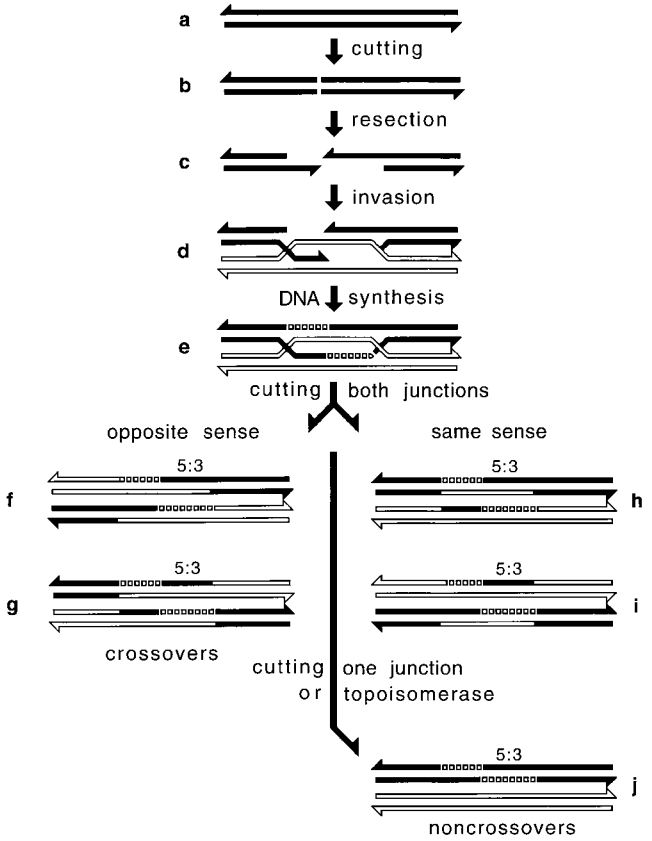

Figure 1.—A model for the repair of double-strand breaks (following Szostak *et al.* 1983; Gilbertson and Stahl 1996). After the DSB occurs, strands ending 5′ at the break are resected, and the exposed 3′ ends invade the homologous chromosome (a–d). The invading 3′ ends prime DNA synthesis, replacing the information lost during resection (and providing the opportunity for gene conversion). The resulting joint molecule (e) can be resolved in a number of ways. f and g are crossover resolutions. In f, the left Holliday junction (HJ) has been resolved by cutting the outside (noncrossed) strands and the right HJ has been resolved by cutting the inside (crossed) strands. In g, the opposite resolutions have occurred. In resolution f, the dark segments contributing to heteroduplex remain linked to the dark flanking DNA. In resolution g, the dark segments contributing to each heteroduplex become linked to light flanking DNA. h and i are noncrossover resolutions of the joint molecule effected by resolvase cutting of both HJs. In h, both HJs were resolved by cutting the crossing strands; in i, both HJs were resolved by cutting the noncrossing strands. The noncrossover resolution j can be effected either by cutting one HJ and sliding the other to that position prior to religation or by the action of topoisomerase. HCs, labeled 5:3, are indicated only where they are relevant to our studies, *i.e.*, to the right of the DSB site.

duplex DNA contributes to the pattern of aberrant segregation. If mismatches in heteroduplex escape repair during meiosis, the alleles will segregate from each other during the first mitotic division after meiosis (PMS). MMR during meiosis can have two outcomes. If the invading strand (the broken strand) is excised and then replaced by DNA synthesis, full conversion results (conversion-type repair). On the other hand, if the intact strand of the invaded duplex is excised and replaced, Mendelian segregation is restored (restoration-type repair). Most studies suggest that markers near a DSB site undergo predominantly conversion-type repair, although restoration-type repair has been observed in yeast (Kirkpatrick *et al.* 1998). The relative frequencies of these two kinds of repair are unknown, because restoration of Mendelian segregation would obscure the fact that heteroduplex had been present.

For some genes, the aberrant segregation frequencies of genetic markers vary monotonically along the length of the gene (conversion gradient), with the high end corresponding to the site of a hotspot for meiosis-specific DSBs (Lissouba and Rizet 1960; Siddiqi 1962; Murray 1963; Fogel and Hurst 1967). The slope of the gradient depends on the involvement of MMR enzymes (Detloff *et al.* 1992; Alani *et al.* 1994). When mismatch repair enzymes can act on mismatches generated during the repair process, the gradient is steep. When the mismatch repair system is inactive, either due to mutation of relevant genes (*e.g.*, *MSH2*) or through the use of markers, such as small palindromes, whose mismatches with wild-type DNA are semirefractory to correction (Nag *et al.* 1989), the gradient is less steep (Detloff and Petes 1992; Detloff *et al.* 1992; Reenan and Kolodner 1992; Alani *et al.* 1994). However, not all mutations eliminating MMR appear to eliminate the gradient (*e.g.*, *mlh1*; Hunter and Borts 1997).

Two models have been proposed to explain the dependence of the gradient on MMR. The heteroduplex rejection model, by analogy to prokaryotic antirecombination activities, suggests that MMR proteins recognize most mismatches in nascent heteroduplex and disrupt the heteroduplex so as to exclude the mismatch. As a result, the HJ on that side of the DSB will be resolved on the DSB-proximal side of the marker, allowing Mendelian segregation for the marker (Reenan and Kolodner 1992; Alani *et al.* 1994; Figure 2A). Most yeast markers, which generate well-repairable mismatches ("well-repairable markers"), will so influence junction-resolution points (JRPs), leading to a decreased aberrant segregation frequency relative to markers generating poorly repairable mismatches ("poorly repairable markers"). The observed gradient reflects the likelihood that mismatches will be recognized by the MMR system with consequent effect on the JRPs.

The restoration-conversion model, on the other hand, proposes that the amount of hDNA, and hence the location of JRPs formed during DSBR, is unaffected by MMR functions and that the gradient reflects instead a change in the preferred direction of repair of mismatches (Detloff *et al.* 1992). Near the initiation site, well-repairable mismatches are proposed to be repaired predominantly on the invading strand, resulting in conversion-type repair. As the distance from the initiation site increases, the likelihood that repair is effected by excision of a portion of the intact strand increases, resulting in increasing likelihood of restoration of Mende-

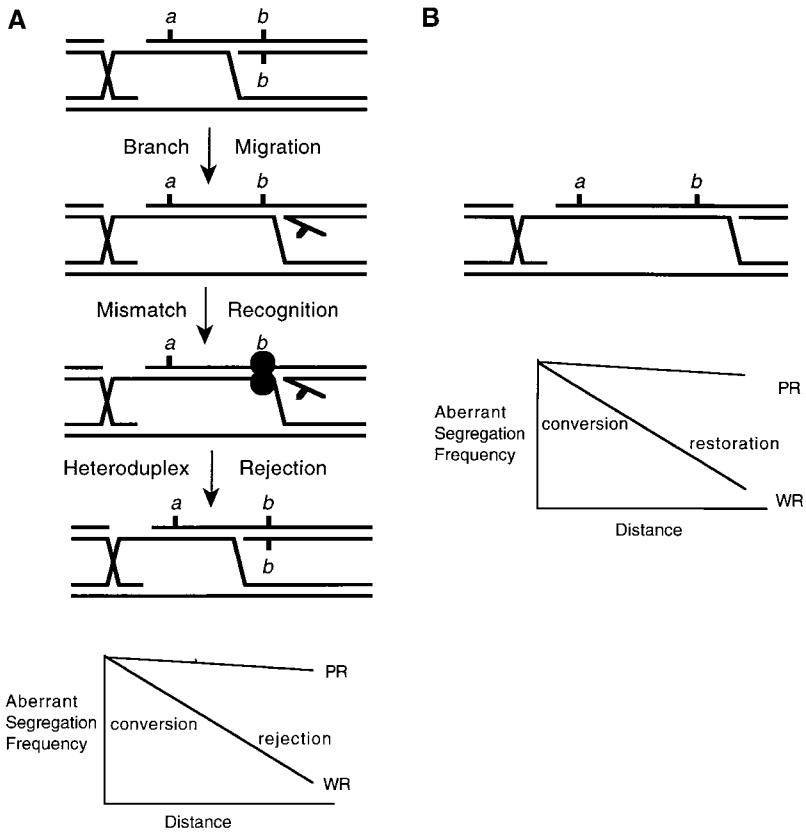

Figure 2.—Models for the origin of the meiotic conversion gradient for markers generating well-repairable (WR) or poorly repairable (PR) mismatches. (A) The heteroduplex rejection model suggests that the amount of heteroduplex formed during DSBR is regulated by the mismatch repair system in response to detected mismatches in heteroduplex. After initial strand invasion, the amount of heteroduplex is thought to increase through branch migration. If branch migration generates a mismatch that can be recognized by the mismatch repair machinery, the direction of branch migration is reversed, shortening the heteroduplex tract. Mismatches formed during strand invasion (mismatch *a*) are largely immune to rejection, while mismatches formed during branch migration (mismatch *b*) are subject to rejection. The observed gradient depends on the likelihood that any particular mismatch will (1) be generated by branch migration and (2) be undone by heteroduplex rejection. (B) The restoration-conversion model proposes that the gradient is due to an increase in the likelihood of restoration-type repair with increasing distance from the initiation site. In the intermediate diagrammed, mismatch *a* is likely to be repaired through excision of information from the broken strand (conversion-type repair), while mismatch *b* is likely to be repaired through excision of information from the bottom strand (restoration-type repair). Note that the restoration-conversion model predicts that JRPs will fall on the DSB-distal side of the recognized mismatch (*b*), while the heteroduplex rejection model predicts that JRPs will fall on the DSB-proximal side of the mismatch.

lian segregation (Figure 2B). Thus, an effective MMR system prevents the detectability rather than the formation of DSB-distal hDNA. Mendelian segregation for a marker within a region of hDNA has been observed at the *HIS1* (Savage and Hastings 1981) and *HIS4* loci (Kirkpatrick *et al.* 1998) of yeast.

These two models make different predictions for the distribution of JRPs near a well-recognized mismatch (Figure 2). The restoration-conversion model predicts that the MMR system will have no effect on the distribution of JRPs. The heteroduplex rejection model, on the other hand, predicts that the amount of hDNA formed during DSBR is regulated by mismatch repair functions, with recognition of mismatches in hDNA altering the JRP with consequent shortening of hDNA around the DSB site. Thus, the effect of the repairability of markers on the distribution of JRPs around a DSB site can be used to distinguish between these two models. If, at a given site, a well-repairable mismatch shortens the region of hDNA more often than does a poorly repairable mismatch, the heteroduplex rejection model is supported. On the other hand, if crosses with well- and poorly repairable mismatches show similar distributions of JRPs, the restoration-conversion model is supported.

The experiments described herein were designed (1) to determine the effect of the MMR system on the distribution of JRPs to one side of a well-characterized DSB hotspot in a subclass of the data, and (2) to reveal the presence or absence of restoration-type repair in that subclass. Our results indicate that the presence of a well-repairable marker at the low end of the *HIS4* conversion gradient causes a shortening of hDNA tracts, and they show no evidence for restoration-type repair in the examined subclass. Whereas the emphasis of this article is on heteroduplex rejection, our data provide compelling, albeit indirect, evidence for restoration-type repair in a different subclass of tetrads as predicted by a novel variation of the DSBR model presented in Foss *et al.* (1999). In the appendix, we present evidence that, in addition to the nonreciprocal hDNA shown in Figures 1 and 2, hDNA in yeast is sometimes formed reciprocally on two interacting chromatids.

MATERIALS AND METHODS

Genetic analysis: Standard procedures and media were used for vegetative growth of yeast (Sherman 1991). The following modified sporulation regimen was used: Diploid yeast strains were grown to saturation in YEPD broth. The cells were pelleted, washed, and diluted 1:10 into liquid SPM (Kassir and Simchen 1991) supplemented with the 20 amino acids at one-fifth the concentration recommended for yeast growth (Sherman 1991). The sporulation cultures were incubated

for 5 days in a shaking water bath at 18°, by which time the cultures had reached maximal sporulation. Tetrads were treated with Glusulase (New England Nuclear, Boston) and dissected onto 2× YEPD plates. After 2–3 days, they were replica plated to appropriate omission media to follow the segregation of nutritive markers. Data were used from four-spore-viable tetrads only, which comprised ~60% of tetrads dissected.

Allelism testing: The *HIS4* gene shows intragenic complementation, with three complementation groups (*HIS4A*, *HIS4B*, and *HIS4C*). Thus, segregation of *his4* markers in heteroallelic crosses can be followed by complementation analysis. *his4-IR9* is an in-frame insertion in *HIS4A* (*his4a HIS4B HIS4C*). *his4-3133* and *his4-713* are mutations in *HIS4C* (*HIS4A HIS4B his4c*). Spore colonies from heteroallelic crosses were replica printed to plates spread with lawns of **a** and α tester strains, either *his4a his4b HIS4C* (PD21 and PD68 or KY56 and KY57) or *HIS4A HIS4B his4c* (KY32 and KY33 or KY42 and KY43). In the case of PD21 and PD68, the tester strains were spread on YEPD plates. After the spore colonies were replica printed, the plates were incubated overnight to allow mating and then were replica printed to SD-his to test for complementation. All of the other tester strains used were *ade2 ADE6*, while the experimental strains were *ADE2* a*de6*. This allowed direct selection of diploids on SD-ade plates. Selected diploids were then replica printed to SD-his plates.

Control experiments established that spore colonies would mate efficiently with lawns of tester strains on SD-his plates, allowing determination of intragenic complementation without an intermediate mating step. Thus, partway through this analysis we switched to direct assay of complementation on SD-his plates spread with lawns of testers.

Sectored colonies: The markers *his4-3133* and *his4-IR9* are small palindromic insertions. When present in heteroduplex, the resulting mismatches are poorly repairable by the MMR system, and crosses carrying these markers frequently produce spores that show PMS at *HIS4*. For crosses carrying only one marker, PMS at *HIS4* can be detected by direct replica printing of spore colonies to SD-his plates. Heteroallelic crosses require additional replica printing. Any colony that could not be clearly assigned as sectored or nonsectored was streaked to a minimum of 20 single colonies and retested. In addition, randomly selected tetrads were streaked to a minimum of 20 single colonies and retested to monitor our ability to detect sectored colonies. Sectored colonies were correctly identified with >95% accuracy.

Yeast strains: All experimental strains (Figure 3; Table 1) were derived from AS4 (*MAT*α *trp1 arg4 tyr7 ade6 ura3*) and AS13 (*MAT***a** leu2 ade6 ura3; Stapleton and Petes 1991). These strains have high levels of meiotic recombination at *HIS4* when sporulated at 18°. Strains AS4, AS13, DNY47, PD100, and PD98 were obtained from Tom Petes (University of North Carolina). KY26 was made by replacing the *HIS4* gene in AS4 with the *his4-713* allele through two-step transplacement (Scherer and Davis 1979) with *Spe*I-digested phis4-713 (from G. Fink via T. Petes). KY29 was made by replacing the *HIS4* gene in AS4 with the *his4-3133* allele through two-step transplacement with pPD6 (Detloff *et al.* 1992) cut with *Blp*I. KY44, KY45, KY46, and KY47 were derived from KY26, AS4, DNY47, and PD100, respectively. In each case the parent strain was transformed with a fragment from the plasmid pMW33 (White and Petes 1994) carrying sequences flanking *HIS4* with the *URA3* gene inserted into a *Spe*I site. One-step integration of this fragment places *URA3* 3.7 kb downstream (and CEN-distal) of *HIS4* (see Figure 3). Insertion of *URA3* induced disparity of aberrant segregation frequency in each case, always in favor of the markers present on the chromosome carrying the hemizygous *URA3* insertion. The chromosomal location of the *URA3* insertion had no effect, however, on the distribution of JRPs. For example, KY48 and KY49 differ only in the chromosome carrying the *URA3* insertion, and have an indistinguishable distribution of JRPs to the right of the 5′ DSB site in *HIS4* (data not shown). Therefore, disparity induced by the *URA3* insertion does not intrude on this analysis.

Statistics: A one-tailed test for significance of differences between two frequencies (p_1 and p_2) in Table 3 was with the statistic $T = 2(\theta_1 - \theta_2)(n_1^{-1} + n_2^{-1})^{-1/2}$, where $\theta_1 = \arcsin(p_1^{1/2})$ and $\theta_2 = \arcsin(p_2^{1/2})$ are measured in radians, and n_1 and n_2 are the respective sample sizes. A T value ≥ 2.33 implies a probability ≤ 0.01 that $p_1 > p_2$ due to sampling error alone. The test was suggested by Russ Lande and is based on the arcsin transformation (see, *e.g.*, Sokal and Rohlf 1995).

RESULTS

Experimental rationale: Two approaches were used to distinguish between the heteroduplex rejection model and the restoration-conversion model. First, we tested the prediction of the heteroduplex rejection model that the extent of hDNA, and therefore the distribution of JRPs, varies with the repairability of a mismatch far from the DSB site but potentially within the region of heteroduplex. This model predicts that such a mismatch is excluded from, and thus shortens, the region of heteroduplex, but only if it is subject to mismatch repair. In the second approach, we tested a subclass of tetrads for the prediction of the restoration-conversion model that a well-repairable mismatch within the DSB-distal region of heteroduplex undergoes frequent restoration-type repair. Both approaches require that JRPs be recognizable.

In the complete absence of MMR, JRPs are potentially recognizable in each of the five tetrad types shown generically in Figure 1. If the DNA segments within and outside the region of heteroduplex are genetically marked, the JRPs are represented by the DSB-distal points of separation between previously linked markers. If a mismatch is acted upon by the MMR system, however, the point of separation between previously linked markers does not necessarily represent the JRP, in which case the effects of heteroduplex rejection and restoration-type repair will be indistinguishable from each other. Consider the type f tetrad illustrated in Figure 1. If the MMR system turns the HC to the right of the DSB, for example, into a full conversion (6:2) by causing excision of the black "5:3" segment and replacing it using information from the white segment, the point of separation between black and white still represents the JRP. On the other hand, if the MMR system were to cause excision of the white segment of the HC so as to restore Mendelian segregation of black and white for the affected segment, the JRP would be unchanged, but the point of separation between black and white information will have moved toward the DSB on the left. Thus, the 5:3 mismatch may appear to have triggered

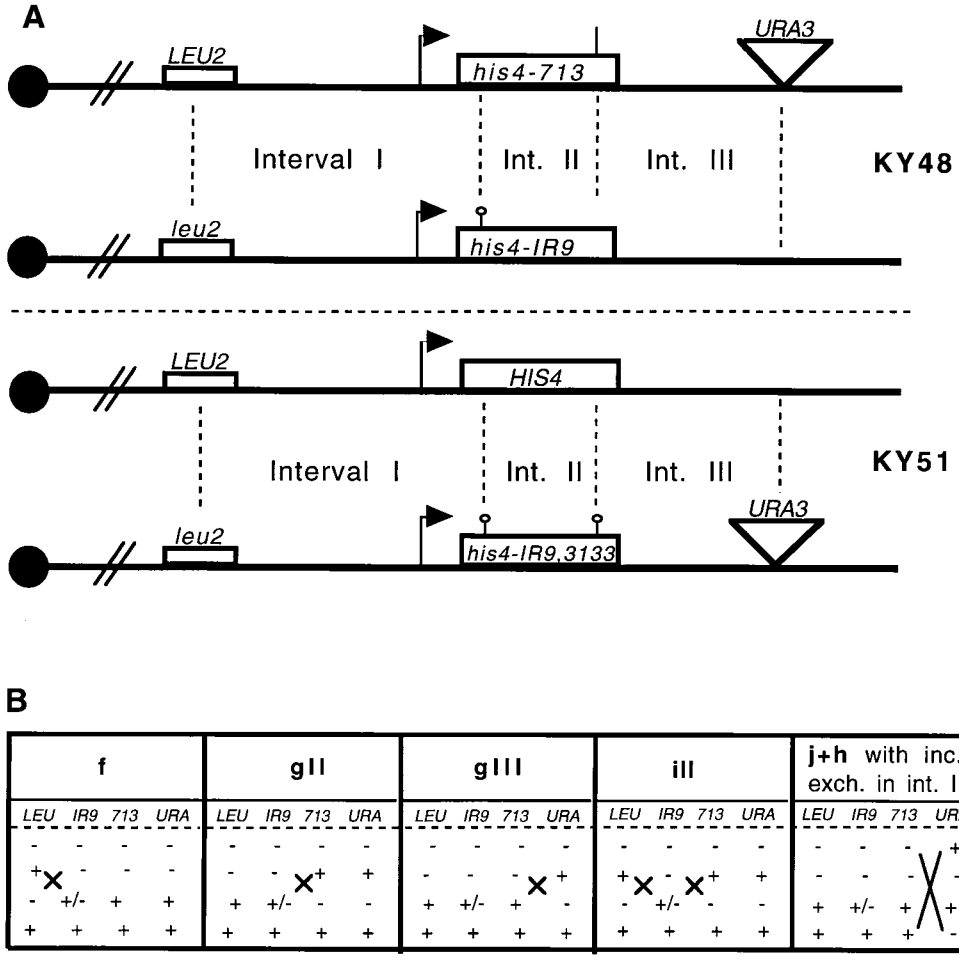

Figure 3.—Experimental systems. (A) Strains KY48 and KY51 are shown, with intervals I, II, and III (not drawn to scale). Arrows indicate the meiosis-specific DSB site in the promoter of *HIS4*, to the left of *his4-IR9*. KY49 differs from KY48 only in the chromosome carrying the *URA3* insertion. In KY52, the *LEU2* chromosome carries the *his4-3133*, and the *leu2* chromosome carries *his4-IR9* and the *URA3* insertion. KY53, KY54, and KY55 each carry a single *his4* marker, *IR9*, *713*, or *3133*, respectively, as well as the *LEU2* and *URA3* markers. Interval I is 23 kb in length, interval II is 1.8 kb, and interval III is 4 kb. Among tetrads that gave Mendelian segregation for all markers and contained crossovers for the flanking markers, *LEU2* and *URA3*, the fraction of exchanges in the three intervals were, respectively, 76, 5.7, and 18%. (B) Tetrads of types f, gII, gIII, iII, and j (or h), showing segregation of markers in meioses of strain KY48, with HC of *his4-IR9*. The point of separation (marked with X) of the minority *IR9* segment from its previously linked, flanking marker(s) is diagnostic of the type of HJ resolution. The tetrad of type j (or h) is shown with a demonstrably incidental exchange in interval III. Note that tetrads of type gIII with Mendelian segregation of *his4-713*, as illustrated, are predicted to occur rarely according to the heteroduplex-rejection model but frequently according to the restoration-conversion model (but see Foss *et al.* 1999).

heteroduplex rejection, while it had in fact undergone restoration-type repair.

Among the five types of HJ resolution depicted in Figure 1, only resolutions of types g and i allow identification of the right-hand JRP independently of whether the MMR system is active. For these types the point of separation between markers represents the JRP whether the MMR system is (1) inactive, (2) causes heteroduplex rejection, or (3) causes restoration-type repair. Note that conversion-type repair of the 5:3 mismatch will move the point of separation between black and white toward the DSB; nevertheless, the presence of the full conversion signals that the JRP had, in fact, occurred on the DSB-distal side of the mismatch. In practice, the identification of tetrad types requires that the region of heteroduplex (marked 5:3 in Figure 1) as well as the spatial relationships between the region of heteroduplex and the flanking markers be identifiable.

Strains constructed for this work are listed in Table 1 and described in Figure 3. The strains have the following features: (1) a hotspot for DSBs located upstream of the HIS4 gene, resulting in levels of aberrant segregation as high as 50% at the 5′ end of *HIS4* (Nag *et al.* 1989; Detloff *et al.* 1991; Nag and Petes 1993; Fan *et al.* 1995; Baudat and Nicolas 1997); (2) a poorly repairable genetic marker, *his4-IR9*, at position +467 (strains KY48, KY49, KY51, and KY52), used for identifying DSB-associated recombination events and for identification of resolution types; (3) either a well-repairable marker, *his4-713* at +2270 (strains KY48, KY49), or a poorly repairable marker, *his4-3133* at +2327 (strains KY51, KY52), near the 3′ end of *HIS4* to register the effects of the MMR system on the distribution of JRPs and to allow identification of JRPs; and (4) genetic markers flanking the *HIS4* gene for identifying types of HJ resolution, JRPs, and exchanges incidental to any DSB that led to aberrant segregation at *his4-IR9*. Strains KY53, KY54, and KY55 (not shown) each have a single *his4* marker to allow determination of conversion frequencies of individual markers. Genotypes of our strains have been confirmed by Southern analysis and tetrad dissection.

Tetrads of all types observed from KY48, KY49, KY51, and KY52 are diagrammed and enumerated in the appendix, Figure A1. Tetrads in which two chromatids

TABLE 1

Yeast strains

Strain	Genotype (or parents)
AS4	*MAT*α *trp1 arg4 tyr7 ade6 ura3*[a]
AS13	*MAT***a** *leu2 ade6 ura3*[a]
DNY47	AS13 *his4-IR9* (18-bp palindromic insertion at +467)[b]
PD100	AS13 *his4-IR9 his4-3133* (18-bp palindromic insertion at +467; 26-bp palindromic insertion at +2327)[c]
PD98	AS13 *his4-3133* (26-bp palindromic insertion at +2327)[d]
KY26	AS4 *his-713* [1-bp insertion (G) at +2270]
KY29	AS4 *his4-3133* (26-bp palindromic insertion at +2327)
KY44	KY26 *Spe*I:*URA3* [*URA3* gene inserted downstream (CEN-distal) from *His4*]
KY45	AS4 *Spe*I:*URA3*
KY46	DNY47 *Spe*I:*URA3*
KY47	PD100 *Spe*l:*URA3*
KY48	DNY47/KY44
KY49	KY46/KY26
KY51	KY47/AS4
KY52	KY46/KY29
KY53	KY46/AS4
KY54	AS13/KY44
KY55	PD98/KY45
	Tester strains[e]
PD21	AS13 *his4-*Δ*29* (*his4a his4b HIS4C*)[c]
PD68	AS4 *his4-*Δ*29* (*his4a his4b HIS4C*)[c]
KY32	*MAT***a** *ade2 his4-713* (*HIS4A HIS4B his4c*)
KY33	*MAT*α *ade2 his4-713* (*HIS4A HIS4B his4c*)
KY42	*MAT***a** *ade2 his4-3133* (*HIS4A HIS4B his4c*)
KY43	*MAT*α *ade2 his4-3133* (*HIS4A HIS4B his4c*)
KY56	*MAT***a** *ade2 his4-*Δ*29* (*his4a his4b HIS4C*)
KY57	*MAT*α *ade2 his4-*Δ*29* (*his4a his4b HIS4C)*

[a] Stapleton and Petes (1991).
[b] Nag and Petes (1991).
[c] Detloff and Petes (1992).
[d] Detloff *et al.* (1992).
[e] Tester strains were used for allelism tests as described. PD21 and PD68 were obtained from Tom Petes' laboratory (University of North Carolina). All other tester strains are segregants from crosses between various lab strains, selected for the presence of desired alleles. Other alleles are uncharacterized.

showed PMS for *his4-IR9* (aberrant 4:4 tetrads) were excluded from our analysis (but see the appendix) as were tetrads wherein aberrant segregation at the two *his4* markers could not be attributed to one DSB. A few additional tetrads were eliminated from some analyses as described in the appendix, Table A1.

For the identification of tetrad types and JRPs, we used only tetrads with a half conversion for *his4-IR9* (HC(*IR9*)). Each such 5:3 tetrad defines a minority marker in the region of heteroduplex, *i.e.*, the marker represented by only three of the eight single strands present in the tetrad. In tetrad types g and i, the right-hand JRP separates the minority marker from the *URA3* marker on the PMS chromatid. In type g tetrads, the minority *his4-IR9* segment remains linked to its flanking DNA on the left. [In the language of previous workers (*e.g.*, Fogel *et al.* 1979; Gilbertson and Stahl 1996), in g tetrads "the exchange is on the right."] In type i tetrads, the minority *his4-IR9* segment is separated from its flanking DNA on both sides of *HIS4*. The markers at the 3′ end of *HIS4* (*his4-713* and *his4-3133*), which serve to register the effects of the MMR system, also serve to locate the JRPs. Tetrads of types g or i have a JRP in interval II if the 3′ marker shows Mendelian segregation and remains linked to its flanking marker on the right; the JRP is in interval III if the 3′ marker shows (1) FC, (2) HC as coconversion with HC(*IR9*), or (3) Mendelian segregation accompanied by separation from its flanking marker on the right.

Not all tetrads with type g or i phenotypes are, in fact, the result of opposite-sense or same-sense HJ resolution, respectively. Although many of the crossovers between the markers flanking *HIS4* result from DSBs in the *HIS4* promoter region, some were initiated elsewhere, while some were initiated at the *HIS4* promoter region by a second DSB that did not result in aberrant segregation (Porter *et al.* 1993). If such an "incidental exchange" involves the chromatid with PMS at *his4-IR9*, it can, for example, cause a tetrad of resolution type j to mimic one of type f or type g, depending on whether the exchange occurs in interval I or in intervals II or III, respectively. The method by which we corrected our data for incidental exchanges is described in the appendix.

Aberrant segregation frequencies: Aberrant segregation frequencies for the *his4* markers present in each of the strains used in this experiment are shown in Table 2. The aberrant segregation frequency of a well-repairable marker at the 3′ end of *HIS4* (KY54; *his4-713*, +2270) is lower than that of a poorly repairable marker in approximately the same place (KY55; *his4-3133*, +2327; 16.6% *vs.* 33.7%), in concordance with earlier studies. As observed by Detloff and Petes (1992), the aberrant segregation frequency of *his4-3133* is lower in heteroallelic crosses (KY51 and KY52) than in a cross with no other *HIS4* markers segregating (KY55; 25.9% aberrant segregation *vs.* 33.7%; $\chi^2 = 7.50$, $P < 0.01$). This indicates that the presence of the upstream marker reduced the formation of hDNA at the downstream marker, a result predicted by the heteroduplex rejection model (Alani *et al.* 1994) if palindromes are sometimes recognized by the MMR system (as implied by the fact that 25–30% of the conversions of such markers are full conversions).

The *HIS4* aberrant segregation frequencies in Table 2 refer to all four-spore-viable tetrads from each cross. The distribution of JRPs and incidental exchanges, however, was determined from those four-spore-viable tetrads that had Mendelian segregation of the markers

TABLE 2

Aberrant segregation frequencies

Strain	Marker	Aberrant segregation (%)	PMS/Ab. seg. (%)	Number of tetrads
KY48	*his4-IR9*	35.0	77	1282
	his4-713	14.9	0.2	
KY49	*his4-IR9*	36.5	74	422
	his4-713	16.8	0	
KY51	*his4-IR9*	34.6	72	1093
	his4-3133	25.9	73	
KY52	*his4-IR9*	39.9	70	609
	his4-3133	25.9	70	
KY53	*his4-IR9*	34.7	81	271
KY54	*his4-713*	16.6	0	259
KY55	*his4-3133*	33.7	73	306

Aberrant segregation frequencies are from all tetrads with four viable spores. PMS/Ab. seg. is the fraction of aberrant segregation tetrads with at least one spore showing postmeiotic segregation. Number of tetrads is the number of four-spore-viable tetrads analyzed in each cross.

flanking *HIS4* (appendix, Figure A1). The aberrant *HIS4* segregation frequencies in tetrads with Mendelian segregation of flanking markers were not significantly different from those found in the full data set.

Distribution of junction-resolution points: To determine the effect of MMR on the distribution of JRPs, we identified JRPs at the 3′ end of *HIS4* (Figure 3) among tetrads with HJ resolution of types g and i from two sets of diploid strains. Strains KY48 and KY49 each have a well-repairable marker at the 3′ end of *HIS4* (*his4-713*), but they differ in the arrangement of flanking markers (Figure 3). The distributions of recombinant tetrad types in the two crosses are statistically indistinguishable (data not shown), so the results from the two crosses have been pooled for further analysis. KY51 and KY52, which both have a poorly repairable marker at the 3′ end of *HIS4* (*his4-3133*), differ in the arrangement of flanking markers and in the arrangement of *HIS4* markers as well (Figure 3). The distributions of recombinant tetrad types in these two crosses are also statistically indistinguishable (data not shown). Thus, the data from KY51 and KY52 have also been pooled. The data, collected from the tetrad classifications in Table A1 of the appendix, are presented in Table 3. Values for tetrads of type g with JRPs in intervals II and III are the observed numbers of type g tetrads of each category and, parenthetically, for that number corrected for incidental exchanges. Similarly, values for tetrads of type i in each category are given for the observed number of type i tetrads and for that number corrected for incidental exchanges. Tetrads that are ambiguously g or i (called g/i) are also included. Note that in KY48 and KY49, 45% of JRPs in tetrads of types g + i + g/i ended in interval II. In KY51 and KY52, however, only 23% of heteroduplexes in such tetrads ended in interval II. These results support the view that the distribution of JRPs in KY48 and KY49 differs from that in KY51 and KY52 as expected for the heteroduplex rejection model. For both the corrected and uncorrected data, the *P* values are <0.01 (see materials and methods).

Restoration-type repair: A specific and sensitive test of the restoration-conversion model focuses on the prediction that tetrads whose JRPs are demonstrably in interval III show Mendelian segregation for the well-repairable 3′ marker. Accordingly, we examined strains KY48 + KY49 and strains KY51 + KY52 for the frequencies of Mendelian segregation of the 3′ marker in tetrads of type gIII, of type iIII, and of the (g/i)III type. The derivation for this frequency expected on the basis of the restoration-conversion model is described below.

Using data from KY51 + KY52 (Table 4, corrected data), we estimate the probability that a region of het-

TABLE 3

Distribution of JRPs

	No. in interval		% in interval	
Cross/type	II	III	II	III
KY48 + KY49				
g	16 (17)	37 (31)		
i	13	12		
g/i	8	2		
Total	36 (37)	51 (45)	41 (45)	59 (55)
KY51 + KY52				
g	8 (7)	39 (36)		
i	4	7 (6)		
g/i	2	1		
Total	14 (13)	47 (43)	23 (23)	77 (77)

Tetrads were assigned to type as described in Table A1. The numbers in parentheses have been corrected for incidental exchanges as described therein. The g/i types are not subject to correction, because the incidental exchanges (all in interval I) convert g to i and vice versa.

TABLE 4

Repairability and segregation

Cross/type	Interval	Segregation of 3′ marker			
		4:4	FC	HC	Total
KY48 + KY49					
j + h	—	104 (128)	16 (22)	0	120 (150)
f	—	87 (67)	11 (6)	0	98 (73)
g	II	16 (17)	0	0	16 (17)
	III	12 (7)	25 (24)	0	37 (31)
i	II	12	0	0	12
	III	3	9	0	12
g/i	II	8	0	0	8
	III	0	2	0	2
Total		242	63	0	305
KY51 + KY52					
j + h	—	62 (71)	5	62 (74)	129 (150)
f	—	36 (27)	5	41 (34)	82 (66)
g	II	8	0	0 (−1)	8 (7)
	III	3	9	27 (24)	39 (36)
i	II	4	0	0	4
	III	0	0	7 (6)	7 (6)
g/i	II	2	0	0	2
	III	1	0	0	1
Total		116	19	137	272

Tetrads were assigned to type as described in Table A1. The numbers in parentheses have been corrected for incidental exchanges as described therein.

eroduplex initiated at the *HIS4* DSB site reaches the 3′ marker. Among tetrads that are HC(*IR9*), 57.4% (156/272) have non-Mendelian segregation for the 3′ marker. If the 3′ poorly repairable marker is not undergoing appreciable restoration-type repair, this value will approximate the real extent of right-hand hDNA in the heteroallelic crosses. If it is undergoing restoration, it will represent a minimal value of the fraction of HC(*IR9*) tetrads that have JRPs in interval III. According to the restoration-conversion model, tetrads that are HC(*IR9*) will have JRPs in interval III with a constant frequency (here, at least 57.4%), irrespective of the repairability of the 3′ marker. Repair of a 3′ mismatch can result in either full conversion or restoration of Mendelian segregation of the 3′ marker. Out of 305 tetrads from KY48 + KY49 with a half conversion for *his4-IR9*, none showed HC for *his4-713*, but 20.7% (63/305) showed FC for that marker. Within the restoration-conversion model, this implies that *his4-713* undergoes restoration-type repair in at least 57.4% − 20.7% = 36.7% of all HC(*IR9*) tetrads, and that among HC(*IR9*) tetrads from KY48 + KY49 with JRPs in interval III, no more than 20.7%/57.4% = 36.0% should show full conversion, while at least 36.7%/57.4% = 64.0% should show Mendelian segregation for the 3′ marker.

For crosses KY48 and KY49, tetrads of types gII and gIII, iII and iIII, and (g/i)II and (g/i)III were characterized for (1) Mendelian segregation (2) FC, or (3) HC of the 3′ marker. The results, shown in Table 4, give no evidence of restoration-type repair. While KY48 + KY49 tetrads with JRPs in interval III were predicted to show at least 64.1% 4:4 segregation and not >35.9% FC for the 3′ marker, we observed that only an estimated 7 out of 31 tetrads of type gIII, 3 out of 12 tetrads of type iIII, and 0 out of 2 of type (g/i)III enjoyed Mendelian segregation for the 3′ marker. Thus, whereas 28.8 of 45 tetrads were expected to show 4:4 segregation, only 10 of 45 did so. These results demonstrate that restoration-type repair within the 3′ end of a region of heteroduplex is infrequent, at least in the classes of tetrads examined ($P < 0.001$, taking 64% as based on infinite sample size).

In an accompanying article, Foss *et al.* (1999) offer a modified DSBR theory driven by data from the *ARG4* locus of *S. cerevisiae*. This theory predicts that conversion-type repair will predominate in g tetrads, while restoration-type repair will predominate in f tetrads. To test this theory, we collected the data from both kinds of tetrads (Table 4). Examination of the f and g tetrad types for relative frequencies of HC, FC, and Mendelian segregation of the 3′ marker revealed the following: The g-type tetrads, which account for 39.6% of all g + f tetrads, account for 75.0% of those with FC of the 3′ marker, but for only 27.1% of those with Mendelian segregation of the 3′ marker. These data demonstrate a relationship between the sense of HJ resolution and

the direction of mismatch repair (full conversion *vs.* restoration) that is predicted by the model in Foss *et al.* (1999).

DISCUSSION

To distinguish between the heteroduplex rejection model (Alani *et al.* 1994) and the restoration-conversion model (Detloff *et al.* 1992) for the origin of the meiotic conversion gradient, we examined hDNA formed during repair of DSBs in the *HIS4* gene in crosses with a well-repairable marker present at the 3′ end of *HIS4*, and compared that with the hDNA in isogenic crosses with a poorly repairable marker at the 3′ end of *HIS4*. We find that the distribution of JRPs is affected by the repairability of the 3′ marker—the presence of a well-recognized mismatch results in relocation of the JRPs as predicted by the heteroduplex rejection model but not by the restoration-conversion model. In addition, we found little evidence for restoration-type repair in the subclass of tetrads that should, according to simple expectations of a restoration-conversion model, manifest Mendelian segregation of well-repairable marker *his4-713* >60% of the time.

Heteroduplex rejection is supported by our data and is consistent with prior studies of recombination at *HIS4* (Detloff and Petes 1992; Detloff *et al.* 1992; Alani *et al.* 1994) and with studies of conversion gradients in other organisms. For example, a conversion gradient is seen at the *b2* locus of *Ascobolus immersus* (Rossignol *et al.* 1984). In one study, well-repairable markers in *b2* reduced the aberrant segregation frequency of downstream markers, indicating that heteroduplex rejection may occur in this organism as well (Nicolas and Rossignol 1983).

We envision hDNA formation as a two-step process. First, the 3′-ended single strands arising at the DSB invade an intact donor duplex, generating asymmetric hDNA regions surrounding the DSB (Figure 1). This invasion is catalyzed by RecA-like proteins (*e.g.*, Rad51, Dmc1). Although a certain length of perfect homology is required for successful strand invasion, studies in yeast and *E. coli* indicate that this length is only 20–30 bp (Shen and Huang 1986; Datta *et al.* 1997) and the length of the invading single strand is thought to be ~600 nucleotides (Sun *et al.* 1991). Thus, a single mismatch ~500 bp from the initiation site is unlikely to affect the ability of RecA-like proteins to catalyze strand invasion.

Once hybrid DNA has been formed, RecA-like proteins or proteins catalyzing directed branch migration (*e.g.*, a homolog of *E. coli*'s RuvAB) can catalyze assimilation of the invading strand, progressively displacing the homologous strand of the invaded duplex. This strand exchange may switch from an asymmetric to a symmetric mode (Cunningham *et al.* 1980; West *et al.* 1983). Continued strand exchange after the 3′-ending ssDNA tail is fully incorporated will displace the 5′-ending strand from the invading duplex, which can then form hDNA with the displaced strand from the invaded chromatid. This will generate symmetric hDNA (appendix, Figure A2). Alternatively, the displaced 5′-ending strand could be degraded by a single-strand-specific exonuclease, resulting in continued formation of asymmetric hDNA.

We, like others (Alani *et al.* 1994), envision heteroduplex rejection to occur during strand exchange rather than strand invasion. Results of *in vitro* studies with *Escherichia coli* MMR proteins (Worth *et al.* 1994) indicate that MutS can recognize mismatches in the context of the RecA filament and block extension of heteroduplex in a strand-transfer reaction. We presume a similar activity for MutS homologs (*e.g.*, Msh2 or Msh6) in yeast.

A number of mechanisms for heteroduplex rejection can be envisioned. Purified Msh2 protein has been shown to bind HJs *in vitro* (Alani *et al.* 1997). Thus, it is possible that an interaction between MutS homologs bound to mismatches in the recombination intermediate and a Msh2-bound HJ brings about reverse branch migration of the HJ, disassembling the mismatch-containing heteroduplex. Similarly, "activated" MutS homologs may interact with enzymes catalyzing branch migration, bringing about reverse branch migration leading to heteroduplex rejection, or may interact with MutS homologs not involved with mismatch repair such as Msh4/Msh5. Alternatively, simple binding of MutS homologs to a mismatch present in a RecA-like filament may be sufficient to destabilize the filament, resulting in disruption of further strand assimilation and local disassembly of heteroduplex. The data in this article neither implicate MutS homologs nor distinguish among these possibilities. However, in the appendix we present evidence that branch migration, one of the requirements for heteroduplex rejection, occurs in yeast.

To contribute to the conversion gradient, heteroduplex rejection must increase with increasing distance from the initiation site. Thus, we hypothesize that yeast MutS homologs, like *E. coli*'s MutS, translocate bidirectionally along DNA after binding to a mismatch (Allen *et al.* 1997). In *E. coli*, this translocation continues until a signal that can direct mismatch repair is encountered. In yeast, translocation may occur until a signal that can direct either mismatch repair or heteroduplex rejection is encountered. For mismatches near the initiation site, the 3′ end of the invading strand is typically encountered first. This strand discontinuity serves to direct mismatch repair on the invading strand. When MutS homologs translocate away from a mismatch far from the initiation site, they typically encounter the nearby HJ before they encounter a signal that can direct mismatch repair. In this instance, branch migration is reversed, leading to heteroduplex rejection.

Although about half of aberrant segregation events

at the 5′ end of *HIS4* have been attributed to the activity of the DSB site at the 5′ end of *HIS4* (Detloff *et al.* 1992), other studies have indicated that some recombination events at *HIS4* are likely to result from initiations occurring at other DSB sites located on both sides of *HIS4* (White and Petes 1994). An indication of the relative contributions of sites on the two sides of *HIS4* with respect to our data can be obtained from the crosses with poorly repairable markers at both ends of the gene (KY51 + KY52). We found 119 tetrads with HC at the 5′ marker and normal segregation at the 3′ marker, and 24 tetrads with HC at the 3′ marker and normal segregation at the 5′ marker (appendix, Figure A1). These data imply that ~17% of hDNA at *HIS4* is initiated at the 3′ end. Our conclusion that heteroduplex rejection occurs depends on the identification of tetrads with HC of *his4-IR9* and Mendelian segregation of *his4-713*. A DSB occurring at the 3′ end of *HIS4* is unlikely to generate such tetrads because *his4-713* will be near the DSB and thus will be likely to enjoy conversion-type repair when present in heteroduplex.

In view of our observation that the presence of a DSB-proximal marker reduced the frequency of aberrant segregation of a more DSB-distal marker, a DSB site downstream of *HIS4* could, in principle, compromise our analysis. If many events leading to aberrant segregation of *his4-IR9* were initiated at a downstream DSB whose activity was altered by the nature of markers at the 3′ end of *HIS4*, the aberrant segregation frequency of *his4-IR9* should be affected by the presence or nature of markers at the 3′ end of *HIS4*. We found, however, that the aberrant segregation frequency of *his4-IR9* is unaffected by the presence of either well- or poorly repairable markers at the 3′ end of *HIS4* (Table 2). Moreover, the distribution of incidental exchanges is the same whether the marker at the 3′ end of *HIS4* is well or poorly repairable (Table 2). Many of such incidental exchanges are likely to have resulted from DSBs at the 5′ and 3′ ends of *HIS4*. If the activity of the 3′ DSB site were affected in a marker-specific fashion by insertions at the 3′ end of *HIS4*, the distribution of incidental exchanges would be affected as well. Thus, we think it unlikely that events initiating away from the 5′ *HIS4* DSB site(s) interfere with our analyses.

In this article we have focused on the detection of and possible mechanisms for heteroduplex rejection. However, our results and those of other workers (Kirkpatrick *et al.* 1998) also provide evidence for restoration-type repair, as predicted by the model presented in Foss *et al.* 1999. Specifically, the model predicts that restoration-type repair occurs, but does so primarily in tetrads of types f and j + h rather than g and i. This implies that a reduction in MMR efficiency should unmask more restorations, *i.e.*, cause a greater shift from 4:4 to 5:3 segregations among type f than among type g or i tetrads, on the basis of the reasonable assumption that the contribution to such a shift from reduced heteroduplex rejection is independent of subsequent junction resolution.

The model predicts reduced MMR to cause an increase, stemming from three distinct sources, in the total frequency of aberrant segregation. The first source reflects the minimal restoration documented in tetrads of types gIII + iIII, which, by definition, have escaped heteroduplex rejection. These tetrads, therefore, are expected to show a minimal increase in aberrant segregation in response to reduced MMR. The same minimal increase is expected in the class of all tetrads of types g + i, but in these tetrads a second source, reduced heteroduplex rejection (Table 3) in response to reduced MMR, should cause an additional increase in aberrant segregations. Finally, reduced MMR in tetrads of type f is expected to reveal not only heteroduplex rejection comparable to that found in types g + i, but also restoration-type repair in excess to that found in types g + i. The data in Table 4 support these predictions, showing a minimal (1.2–1.3×) increase for tetrad types gIII + iIII, a 1.6-fold increase for all tetrads of types g + i, and a 7.2-fold increase in aberrant segregations among type f tetrads in response to reduced MMR (see also Foss *et al.* 1999). These results indicate a higher frequency of restoration-type repair among type f than among type g tetrads and suggest a substantial contribution to the conversion gradient by restoration-type repair.

The occurrence of heteroduplex rejection is supported by three observations: (1) The presence of a marker near the 5′ end of *HIS4* reduces the rate of aberrant segregation of a marker near the 3′ end; (2) a well-repairable marker has a higher frequency of JRPs on its 5′ side than does a poorly repairable marker at approximately the same site; and (3) replacement of a well-repairable marker by a poorly repairable marker near the 3′ end of *HIS4* results in an increase in aberrant segregation in a class of tetrads that are demonstrably poor in restoration.

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APPENDIX: TETRAD DATA AND ABERRANT 4:4's

We report patterns of segregation for four markers from diploid strains KY48, KY49, KY51, and KY52 and examine the class of tetrads in which two chromatids show PMS for a given marker (ab4:4's) for evidence of symmetric hDNA.

Segregation patterns: Among four-spore-viable tetrads manifesting normal segregation for the flanking markers, 309 different patterns were found for the segregation of the four markers. In Figure A1 each of these

patterns is presented as four rows of four circles, with each row representing one of the four spores in the tetrad and each circle indicating the parental origin of the allele. Thus, solid (or open) circles can represent either the presence or absence of a mutant marker, depending on the strain in question. This was done to allow strains with different configurations of markers to be represented in the same figure (see Figure A1, legend). Half-solid circles represent spores that gave rise to sectored colonies (PMS) for the marker in question [*e.g.*, tetrad class 1, wherein *his4-IR9* is a HC, or tetrad class 164 (ab4:4), wherein two chromatids manifest PMS for a given marker]. For each class of tetrads, Table A1 lists our interpretation of its mode of joint molecule resolution as well as the locations of the right-hand JRP and of any incidental exchanges (see below). The existence of incidental exchanges, those exchanges not dependent on the DSB that initiated the HC at *his4-IR9*, implies that some of these tetrads are mistyped with regard to mode of resolution. Before correction for incidental exchanges, there were 180 f, 100–113 g, 249 (j + h), and 35–48 i tetrads assignable as described in Table A1.

Incidental exchanges: Exchanges that are incidental to the recombination event that gave rise to 5:3 segregation at *IR9* can result in misclassification of a tetrad. Such misclassification will result only when the incidental exchange involves the PMS chromatid. For each such exchange there is expected to be a detectable incidental exchange, which does not involve the PMS chromatid. Table A1 records detectable incidental exchanges. In Tables 3 and 4, the data corrected for incidental exchanges were derived by adjusting the numbers of each tetrad type according to the number of observed, detectable exchanges that would lead to misclassification had they been undetectable. For instance, a j + h tetrad with an incidental exchange in interval II would create a gII tetrad if the exchange involved the PMS chromatid. Thus, a number equal to the number of observed incidental exchanges involving j + h tetrads was added to the j + h type and subtracted from the gII type. See Table A1 for additional explanation.

Aberrant 4:4 segregation: We used a subclass of our data, tetrads with aberrant 4:4 segregation for *his4-IR9* and/or *his4-3133*, to look for evidence of symmetric hDNA. Aberrant 4:4 tetrads (ab4:4's) have one spore of each parental genotype and two spores that show PMS of the marker (Figure A2A). For poorly repairable markers, ab4:4 tetrads are expected to arise due to two homologs each receiving a DSB at the same hotspot (Figure A2B). The interesting ab4:4's, however, are those that might arise in a joint molecule initiated by a single DSB as proposed by models for meiotic recombination that feature outward migration of HJs (Figure A2C). Although classical studies of meiotic recombination in *S. cerevisiae* revealed few ab4:4's (reviewed in Szostak *et al.* 1983), they are seen more frequently in other fungi (*e.g.*, *Ascobolus immersus*; Rossignol *et al.* 1979) as well as in yeast when mismatch repair is compromised (Nag *et al.* 1989; Alani *et al.* 1994). In *S. cerevisiae*, the observed frequency of ab4:4's is approximately equal to the square of the frequency of HC tetrads. Thus, because dual initiations could quantitatively account for all tetrads with ab4:4 segregation, any evidence for the existence of symmetric hDNA would need to be of a qualitative nature.

Among noncrossover ab4:4 tetrads, segregation patterns would be identical whether they had been initiated by one or by two DSBs. Moreover, if symmetric hDNA were formed, we would not expect to find it frequently among noncrossovers because they are resolved predominantly in a manner that would eliminate both mismatches, regenerating homoduplex DNA (type j). In contrast, tetrads with a single crossover allow ab4:4's derived from one *vs.* two DSBs to be distinguished, provided that all ab4:4's arising from two DSBs do so as pictured in Figure A2B (no *ménage a trois* and no tit-for-tat). In single-exchange ab4:4's so derived, three chromatids should show evidence of PMS and/or reciprocal exchange. Conversely, in ab4:4's resulting from a single DSB, the DSB-related exchange should involve only the two heteroduplex-containing chromatids (Figure A2). Thus, the recovery of a significant excess of ab4:4 tetrads with a two-chromatid exchange would be diagnostic of symmetric heteroduplex initiated by a single DSB.

Our data (Table A2) indicate that exchange among 62 ab4:4 tetrads with a single exchange involved the two PMS chromatids 23 times. These tetrads could indicate symmetric hDNA as in Figure A2C, or they could represent incidental exchange occurring in noncrossover tetrads derived from two DSBs. The frequency of demonstrably incidental exchanges allows us to distinguish

Figure A1.—All four-spore-viable tetrads from heteroallelic crosses without aberrant segregation of either flanking marker (*LEU2* or *URA3*) are graphically represented. Each row of four circles describes the constitution of a meiotic spore with respect to *LEU2* (first circle), *his4-IR9* (second circle), 3′ *his4* marker (third circle), and *URA3* (fourth circle). For each cross, the meaning of the solid circles is as follows: KY48—Leu$^+$, *IR9*$^+$ (WT information at the *IR9* site), *713*$^-$ (mutant information at the *713* site) Ura$^+$; KY49—Leu$^+$, *IR9*$^+$, *713*$^-$, Ura$^-$; KY51—Leu$^-$, *IR9*$^-$, *3133*$^-$, Ura$^+$; KY52— Leu$^-$, *IR9*$^-$, *3133*$^+$, Ura$^+$. Tetrad classes were grouped as follows: 1–48, HC at *his4-IR9* and no other aberrant segregation; 49–122, HC at *his4-IR9* and aberrant segregation at the 3′ *his4* marker; 49–60 and 93–101, FC of the 3′ *his4* marker wherein both markers segregate in a fashion consistent with a single initiation event; 81–92 and 113–122, co-HC of *his4-IR9* and the 3′ *his4* marker, with segregation patterns consistent with a single initiation event; 289–309, no aberrant segregations; 164–210, ab4:4 segregation of at least one *his4* marker.

Class		KY48	KY49	KY51	KY52
1		23	5	26	15
2		29	7	15	11
3		8	0	2	0
4		3	2	2	0
5		6	3	4	3
6		1	0	0	0
7		2	2	0	2
8		1	0	1	0
9		3	0	0	0
10		1	0	0	0
11		1	2	0	0
12		2	0	0	0
13		1	0	0	0
14		1	0	0	0
15		1	0	0	0
16		1	0	0	0
17		2	0	0	0
18		1	0	0	0
19		1	0	1	1
20		1	0	0	0
21		0	0	1	0
22		0	0	1	0
23		0	0	0	1
24		0	0	0	1
25		0	0	0	1
26		0	0	0	1
27		0	0	0	1
28		0	0	0	1
29		40	8	5	5
30		35	13	4	4
31		1	3	1	3
32		6	1	0	1
33		10	4	3	1
34		4	0	0	0
35		3	1	0	0
36		1	2	0	1
37		0	1	0	0
38		2	0	0	0
39		1	0	0	0
40		0	1	0	0
41		1	1	0	0
42		0	1	0	0
43		1	0	0	0
44		1	0	0	0
45		1	0	0	0
46		1	0	0	0
47		0	0	1	0
48		0	0	0	1
49		1	2	0	2
50		7	1	1	1
51		12	2	0	1
52		1	0	0	0
53		3	0	0	0
54		1	0	0	0
55		3	0	0	0
56		1	0	0	0
57		2	2	0	0
58		0	1	0	0
59		1	0	0	0
60		1	0	0	0
61		1	1	0	0
62		0	1	0	0
63		0	1	0	1
64		1	0	0	0
65		0	0	1	0
66		0	0	1	0
67		0	0	1	0
68		0	0	1	0
69		0	0	1	2
70		0	0	1	0
71		0	0	1	0
72		0	0	1	0
73		0	0	1	0
74		0	0	0	1
75		0	0	0	1
76		0	0	0	1
77		0	0	0	2
78		0	0	0	1
79		0	0	0	1
80		0	0	0	1
81		0	0	19	9
82		0	0	14	5
83		0	0	9	4
84		0	0	4	0

Class		KY48	KY49	KY51	KY52
85		0	0	1	0
86		0	0	1	1
87		0	0	3	0
88		0	0	1	0
89		0	0	1	0
90		0	0	1	0
91		0	0	1	0
92		0	0	0	1
93		4	3	2	1
94		0	3	2	1
95		6	3	5	2
96		2	0	0	0
97		1	0	0	0
98		1	2	0	0
99		1	0	0	0
100		2	0	0	0
101		0	0	1	0
102		0	1	0	0
103		1	1	0	0
104		1	0	0	0
105		0	1	0	0
106		0	1	0	0
107		1	0	0	0
108		1	0	0	0
109		1	0	0	0
110		1	0	0	0
111		0	0	1	0
112		0	0	0	1
113		0	0	16	5
114		0	0	17	2
115		0	0	6	4
116		0	0	3	2
117		0	0	1	0
118		0	0	4	0
119		0	0	1	0
120		0	0	1	1
121		0	0	0	1
122		0	0	0	1
123		6	1	2	2
124		10	5	1	2
125		1	2	0	0
126		0	1	0	2
127		1	0	0	0
128		1	0	0	0
129		10	1	3	15
130		10	5	16	6
131		1	1	0	1
132		3	2	0	3
133		2	1	0	2
134		1	0	0	0
135		0	1	3	0
136		0	1	0	0
137		0	1	1	0
138		0	0	2	0
139		0	0	0	1
140		2	2	1	0
141		9	2	3	0
142		2	0	0	0
143		1	0	1	0
144		1	0	0	0
145		0	1	0	0
146		0	0	0	1
147		10	1	9	2
148		8	2	14	6
149		1	0	0	0
150		1	1	0	0
151		0	0	2	0
152		1	0	0	0
153		0	0	0	3
154		0	0	1	0
155		0	0	1	0
156		0	0	0	1
157		0	0	0	1
158		0	0	0	1
159		0	0	3	2
160		0	0	5	0
161		0	0	0	1
162		0	0	0	1
163		0	0	0	1
164		6	0	1	0
165		1	1	0	0
166		1	0	0	0
167		1	0	0	0
168		3	0	1	0

Class	KY48	KY49	KY51	KY52
169	3	1	1	2
170	2	0	0	0
171	1	0	0	0
172	1	0	0	0
173	3	0	0	0
174	1	0	0	0
175	1	0	0	0
176	1	0	0	0
177	0	1	0	0
178	0	1	0	0
179	0	0	1	0
180	0	0	3	1
181	0	0	2	2
182	0	0	2	0
183	0	0	1	0
184	0	0	2	0
185	0	0	1	0
186	0	0	1	0
187	0	0	1	0
188	0	0	1	0
189	0	0	1	0
190	0	0	1	0
191	0	0	1	0
192	0	0	1	0
193	0	0	1	0
194	0	0	1	1
195	0	0	1	0
196	0	0	1	0
197	0	0	1	0
198	0	0	1	0
199	0	0	0	1
200	0	0	0	1
201	0	0	0	1
202	0	0	0	1
203	0	0	0	1
204	0	0	0	1
205	0	0	0	1
206	0	0	0	1
207	0	0	0	1
208	0	0	0	1
209	0	0	0	1
210	0	0	0	1
211	1	0	0	0
212	1	0	0	0
213	1	0	0	0
214	0	1	0	0
215	1	0	0	1
216	0	1	0	0
217	1	0	0	0
218	2	0	0	0
219	0	1	0	0
220	2	0	0	0
221	1	0	0	0
222	1	0	1	0
223	2	0	0	0
224	1	0	0	0
225	1	0	0	0
226	1	1	0	0
227	0	1	0	0
228	1	0	0	0
229	1	0	0	0
230	1	0	0	0
231	1	0	0	0
232	0	1	0	1
233	1	0	0	0
234	0	2	0	0
235	0	0	1	0
236	0	0	1	0
237	0	0	3	0
238	0	0	1	0
239	0	0	1	0
240	0	0	1	0
241	0	0	1	0
242	0	0	1	0
243	0	0	1	0
244	0	0	1	0
245	0	0	1	0
246	0	0	1	0
247	0	0	0	1
248	0	0	0	1
249	0	0	0	1
250	0	0	0	1
251	0	0	0	1
252	0	0	0	1
253	0	0	0	1
254	0	0	0	1
255	0	0	0	1
256	0	0	0	1
257	0	0	0	2
258	0	0	0	1
259	0	0	0	1
260	0	0	0	1
261	1	1	0	1
262	4	2	0	0
263	13	2	1	2
264	0	1	0	1
265	0	1	0	0
266	0	1	0	0
267	1	0	2	0
268	4	1	2	1
269	9	3	0	0
270	1	0	1	0
271	1	0	0	0
272	0	0	3	3
273	0	0	3	0
274	0	0	1	1
275	0	0	1	0
276	0	0	1	1
277	0	0	1	0
278	0	0	1	0
279	0	0	1	0
280	0	0	0	1
281	0	0	0	1
282	0	0	0	1
283	0	0	0	1
284	0	0	0	1
285	0	0	0	1
286	0	0	0	1
287	0	0	0	1
288	0	0	0	1
289	429	120	366	172
290	198	67	179	94
291	8	4	1	3
292	45	13	49	18
293	2	1	1	6
294	0	1	0	0
295	2	0	1	0
296	3	0	0	0
297	2	0	3	0
298	1	0	0	0
299	0	1	0	0
300	3	0	0	0
301	1	0	0	0
302	3	2	7	1
303	1	0	0	0
304	1	0	0	0
305	0	1	0	0
306	0	0	1	0
307	0	0	0	1
308	0	0	0	1
309	0	0	0	1

Figure A1.—*Continued.*

TABLE A1

Tetrad classification

Class	Type	JRP	3′ marker	Incidentals	KY48 + KY49	KY51 + KY52
1	j + h	—	4:4	—	28	41
2	f	—	4:4	—	36	26
3	g	II	4:4	—	8	2
4	g	III	4:4	—	5	2
5	j + h	—	4:4	I	9	7
	f		4:4	(1)		
6	j + h	—	4:4	III	1	0
	g	III		(III)		
7	i	II	4:4	—	4	2
8	i	II	4:4	I or II	1	1
	g	II		(I)		
9	g	II	4:4	I	3	0
	i	II		(I)		
10 omit	f	—	4:4	II	1	0
	i	II		(II)		
11 omit	j + h	—	4:4	I + III	3	0
	f	—		(I) + III		
	g	III		I + (III)		
12	i	III	4:4	—	2	0
13 omit	j + h	—	4:4	I + III	1	0
	g	III	4:4	I + (III)		
	f	—	4:4	(I) + III		
14 omit	f	—	4:4	III	1	0
	i	III	4:4	(III)		
15	g	II	4:4	(III)	1	0
16 omit	j + h	II	4:4	II + III	1	0
	g	III	4:4	II + (III)		
17	i	II	4:4	(III)	2	0
18	i	II	4:4	III	1	0
19	f	—	4:4	I	1	2
20	g	II	4:4	II	1	0
21	g	III	4:4	I	0	1
	i	III	4:4	(I)		
22	g	II	4:4	III	0	1
23	g	II	4:4	I	0	1
	i	II		(I)		
24 omit	j + h	—	4:4	I + II	0	1
	f	—		(I) + II		
	i	II		(I + II)		
25 omit	i	III	4:4	III	0	1
	f	—		(III)		
	g	III		(I)		
26	i	II	4:4	I + (III)	0	1
27	i	II	4:4	I	0	1
28	i	II	4:4	III	0	1
29	j + h	—	4:4	—	48	10
30	f	—	4:4	—	48	8
31	g	II	4:4	—	4	4
32	g	III	4:4	—	7	1
33	j + h	—	4:4	I	14	4
	f	—		(I)		
34	j + h	—	4:4	III	4	0
	g	III		(III)		
35	i	II	4:4	—	4	0
36	i	II	4:4	I	3	0
	g	II		(I)		
	i	II		II		
37	i	II	4:4	(I)	1	0
	g	II		I		

(*continued*)

TABLE A1

(Continued)

Class	Type	JRP	3′ marker	Incidentals	KY48 + KY49	KY51 + KY52
38	g	II	4:4	III	2	0
39	f	—	4:4	III	1	0
	i	III	4:4	(III)		
40 omit	j + h	—	4:4	II + III	1	0
	g	II		(II) + III		
41	f	—	4:4	I	2	0
42	g	II	4:4	I + (III)	1	0
	i	II		(I + III)		
43	f	—	4:4	II + III	1	0
44	i	III	4:4	II	1	0
45 omit	i	II	4:4	II + (III)	1	0
	f	—		(II + III)		
	i	III		(II + III)		
	g	II		(I + III)		
46	i	II	4:4	(III)	1	0
47	g	II	4:4	(III)	0	1
48	g	III	4:4	I + II	0	1
49	j + h	—	FC	—	3	2
50	f	—	FC	—	8	2
51	g	III	FC	—	14	1
52	g	III	FC	II	1	0
53	j + h	—	FC	I	3	0
	f	—		(I)		
54	j + h	—	FC	III	1	0
	g	III		(III)		
55	i	III	FC	—	3	0
56	g	III	FC	I + II + III	1	0
57 omit	i	III	FC	III	4	0
	f	—		(III)		
	g	III		(I)		
58 omit	f	—	FC	III	1	0
	i	III		(III)		
59	g	III	FC	I	1	0
	i	III		(I)		
60	i	III	FC	I	1	0
61–80	Discontinuous conversion					
81	j + h	—	HC	—	0	28
82	f	—	HC	—	0	19
83	g	III	HC	—	0	13
84	j + h	—	HC	I	0	4
	f	—		(I)		
85	j + h	—	HC	II	0	1
86	j + h	—	HC	III	0	2
	g	III		(III)		
87	i	III	HC	—	0	3
88	g	III	HC	I	0	1
89 omit	i	III	HC	(III)	0	1
	f	—		III		
90	f	—	HC	I	0	1
91 (omit)	j + h	—	HC	I + II + (II)	0	1
	f	—		(I + II + II)		
92	f	—	HC	(II)	0	1
93	j + h	—	FC	—	7	3
94	f	—	FC	—	3	3
95	g	III	FC	—	9	7
96	j + h	—	FC	I	2	0
	f	—		(I)		
97	g	III	FC	(II)	1	0
98	i	III	FC	—	3	0

(*continued*)

TABLE A1

(Continued)

Class	Type	JRP	3′ marker	Incidentals	KY48 + KY49	KY51 + KY52
99	g	III	FC	I	1	0
	i	III		(I)		
100	i	III	FC	(II)	2	0
101	g	III	FC	I	0	1
102–112	Discontinuous conversion					
113	j + h	—	HC	—	0	21
114	f	—	HC	—	0	19
115	g	III	HC	—	0	10
116	j + h	—	HC	I	0	5
	f	—		(I)		
117	j + h	—	HC	III	0	1
	g	III		(III)		
118	i	III	HC	—	0	4
119 omit	i	III	HC	III	0	1
	f	—		(III)		
	g	III		(I)		
120	g	III	HC	I	0	2
121	f	—	HC	III	0	1
122	g	III	HC	II	0	1
123–309	Not HC at *his4-IR9*					

Tetrads were assigned to type on the basis of the smallest number of events (“events” are conversions with or without exchange plus incidental exchanges) required to generate them. When two assignments are possible by this criterion, both are entered. When incidental interval entry is in parentheses, the exchanges have involved the PMS chromatid. Tetrad classes that have two or more incidental exchanges were omitted from the calculations of Tables 3 and 4, as were tetrads in which more than two assignments could be made. Tetrads that are ambiguous were omitted from those calculations as well, with the following exceptions: in Tables 3 and 4 the types called g/i are those tetrads that are ambiguously g or i; tetrads that, because of incidental exchanges, were scored ambiguously as j + h and either f or g were considered to be j + h. There are three factors that argue for that assignment: (1) there are twice as many ways to create a tetrad that looks like a j + h with an incidental exchange as there are to create an f or a g that appears to have had an incidental exchange involving the PMS chromatid; (2) the j + h class is larger than either the f or g class; (3) chiasma interference, if any, will discourage incidental exchanges in the f and g classes. Tetrad classes not used in Tables 3 and 4 are marked “omit.” Except for tetrad class 64, “discontinuous conversion” means that the 5′ and the 3′ *his4* markers were converted in opposite directions.

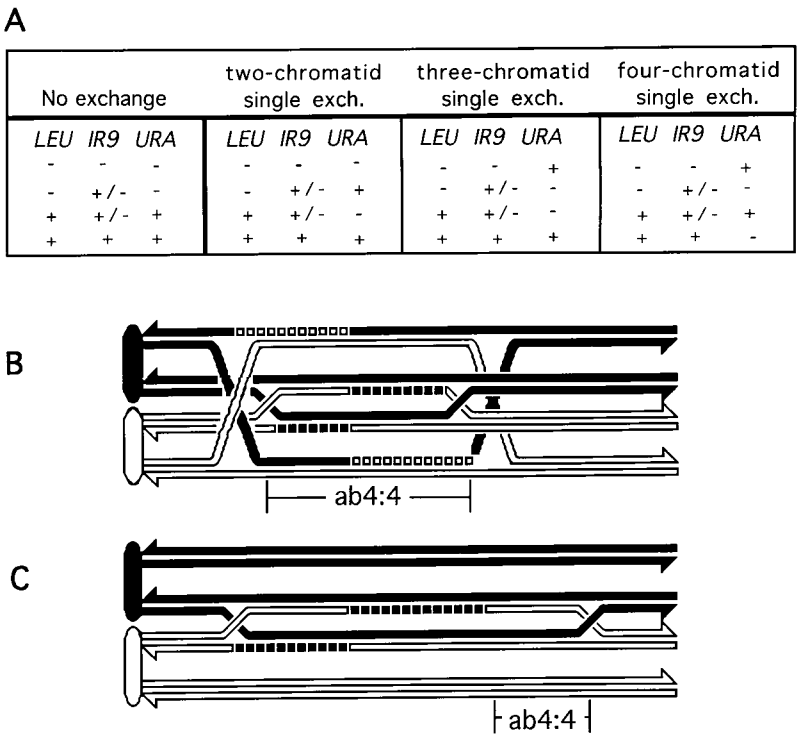

Figure A2.—Aberrant 4:4 tetrads diagrammed for *his4-IR9*. (A) Segregation patterns for ab4:4 tetrads without exchange and with a two-chromatid, three-chromatid, or four-chromatid single exchange, as defined in Table A2. No exchange, two-chromatid exchange, and three-chromatid exchange ab4:4 tetrads are classes 164, 165, and 167, respectively, in Figure A1. (B) A model for aberrant 4:4 segregation resulting from two asymmetric heteroduplexes, with one initiation occurring on each homolog. (C) A model for aberrant 4:4 segregation arising from symmetric heteroduplex.

TABLE A2

Exchanges in ab4:4 tetrads

Marker	No exchange	Single exchange			Multiple exchanges
		Two chromatid	Three chromatid	Four chromatid	
IR9(ab4:4)	19	12	22	1	14
3133(ab4:4)	11	11	15	1	3

All ab4:4 tetrads from strains KY48, KY49, KY51, KY52, and KY55 are shown. A two-chromatid single exchange is a tetrad with a crossover between the two PMS chromatids. A three-chromatid single exchange tetrad has two PMS chromatids and a crossover between one of the PMS chromatids and a non-PMS chromatid. A four-chromatid single exchange tetrad has two PMS chromatids and a crossover between the two non-PMS chromatids.

between these possibilities. If incidental exchanges are randomly distributed among pairs of chromatids in ab4:4 tetrads, we expect 1 tetrad in which the exchange involves the PMS chromatids (two-chromatid single exchange) for every tetrad with a demonstrably incidental exchange, *i.e.*, an exchange between the non-PMS chromatids (four-chromatid single exchange; Table A2). The fact that we recovered only 2 tetrads with a four-chromatid single exchange for 23 tetrads with a two-chromatid single exchange suggests that most of the ab4:4 tetrads with a two-chromatid single exchange were, in fact, derived from a single DSB, implying the existence of symmetric hDNA.

We have considered the possibility that MMR operating on ab4:4's could add, in a misleading way, to the tetrad types upon which we have based our conclusions regarding JRPs and restorations. Although we have no rigorous argument against that possibility, we have failed to find any plausible scenario that could lead to such problems.