The Conversion Gradient at *HIS4* of *Saccharomyces cerevisiae*. II. A Role for Mismatch Repair Directed by Biased Resolution of the Recombinational Intermediate

Henriette M. Foss, Kenneth J. Hillers[1] and Franklin W. Stahl

Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229

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ABSTRACT

Salient features of recombination at *ARG4* of Saccharomyces provoke a variation of the double-strand-break repair (DSBR) model that has the following features: (1) Holliday junction cutting is biased in favor of strands upon which DNA synthesis occurred during formation of the joint molecule (this bias ensures that cutting both junctions of the joint-molecule intermediate arising during DSBR usually leads to crossing over); (2) cutting only one junction gives noncrossovers; and (3) repair of mismatches that are semirefractory to mismatch repair and/or far from the DSB site is directed primarily by junction resolution. The bias in junction resolution favors restoration of 4:4 segregation when such mismatches and the directing junction are on the same side of the DSB site. Studies at *HIS4* confirmed the predicted influence of the bias in junction resolution on the conversion gradient, type of mismatch repair, and frequency of aberrant 5:3 segregation, as well as the predicted relationship between mismatch repair and crossing over.

GILBERTSON and Stahl (1996) drew attention to several properties of recombination at the *ARG4* locus of *Saccharomyces cerevisiae* that are not implicit in the canonical model for meiotic double-strand-break repair (DSBR). Each of these had been reported separately in earlier work at *ARG4* or at other loci or was implied by previous observations. The occurrence of all of them in a single cross encouraged us to rationalize them within a single model. Here we identify deviations from the canonical model and test predictions of a modified model, including those applying to the origin of the conversion gradient, which have been addressed in recent publications (Alani *et al.* 1994; Kirkpatrick *et al.* 1998; and see Hillers and Stahl 1999).

The centerpiece of the canonical DSBR model is a double-Holliday-junction intermediate (Figure 1). This intermediate (joint molecule) arises via invasion by the two overhanging 3′-ended single strands into the same chromatid, followed by DNA synthesis primed by the invading ends. This feature of the model is recommended by its simplicity and by the physical demonstration of some of its salient features (*e.g.*, Schwacha and Kleckner 1995).

The crosses performed by Gilbertson and Stahl (1996) were designed to test central predictions of the canonical DSBR model: (1) Hybrid DNA (hDNA) should be formed on each side of a DSB; (2) this hDNA, detected with appropriate markers as heteroduplex DNA, should be found in separate haploid products of a single meiosis; and (3) in a fraction of such tetrads, the heteroduplex-containing chromatids should be reciprocally recombinant (crossed over) for markers flanking the heteroduplex regions.

To facilitate the testing of these predictions, the crosses of Gilbertson and Stahl (1996) had the following features (Figure 2): (1) Markers were inserted close to and on opposite sides of the DSB site (to reveal the expected heteroduplexes, these markers were small palindromes, which are semirefractory to mismatch repair (MMR) when in heteroduplex with the wild type); (2) one of the two palindromes was in *ARG4*, which permitted phenotypic screening for tetrads manifesting conversion; and (3) auxotrophic markers flanking the *ARG4* region permitted the identification of tetrads in which conversion at the DSB site was accompanied by crossing over.

The data collected by Gilbertson and Stahl (1996) differed from the simple predictions of the canonical model in several ways. First, tetrads that had heteroduplex DNA for both palindromes were found, but few of them showed the heteroduplexes on separate chromatids, and those that did were all crossovers. [Gilbertson and Stahl (1996) noted that their failure to find evidence for steps h and i of the canonical model (Figure 1) is, in fact, a corollary of the long-standing observation that "aberrant 5:3's" are rarely observed in *S. cerevisiae* for the well-documented *ARG4* gene; for review see Szostak *et al.* (1983).] Conversely, among the noncrossover tetrads that were doubly heteroduplex, the two heteroduplexes were invariably located

Corresponding author: Franklin W. Stahl, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229.
E-mail: fstahl@molbio.uoregon.edu

[1]*Present address:* Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305-5329.

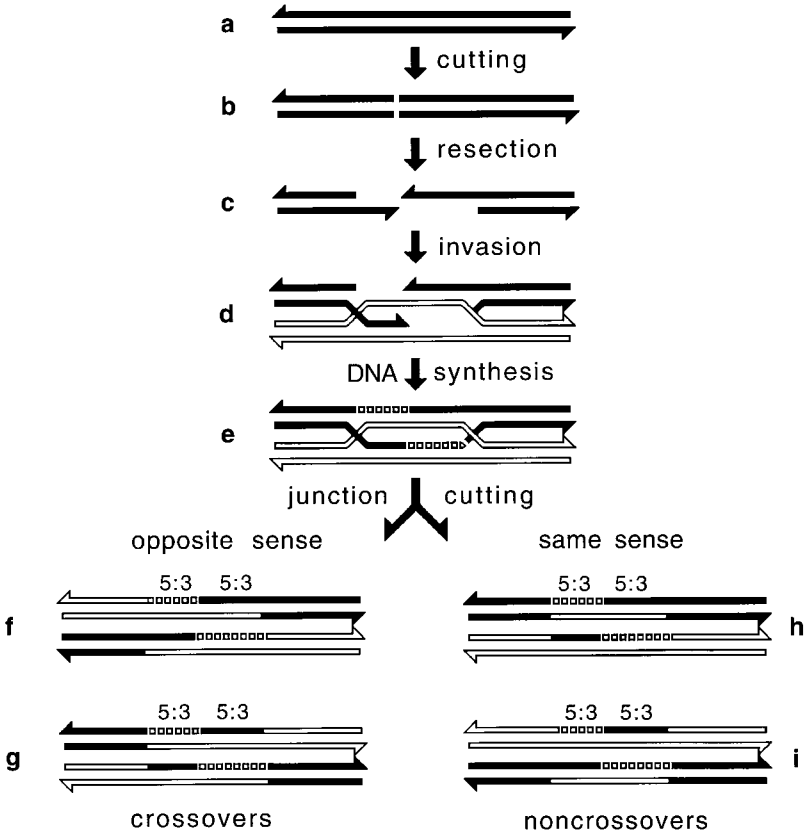

Figure 1.—The canonical DSBR model. A DNA duplex chromatid (a) is cut on both strands (b) and then resected on both sides of the break in the 5′–3′ direction. The resulting 3′-ended overhangs (c) bind a RecA-like protein and invade a homolog chromatid (d). DNA synthesis primed by the invading ends completes the joint molecule (e) in which the two participants are tied together by a pair of Holliday junctions. The joint-molecule intermediate is resolved back to duplexes by resolvase-catalyzed cutting of the junctions. When the junctions are cut in the opposite sense (left), crossovers result. (f) The noncrossing strands were cut at the left junction (vertical cutting), while the crossing strands were cut at the right junction (horizontal cutting). (g) The cutting was horizontal-vertical. When the junctions are cut in the same sense (right), noncrossovers result. (h) The junctions were cut horizontal-horizontal, and in (i) they were cut vertical-vertical. Same-sense resolutions can result in "aberrant 5:3 tetrads," in which each of the noncrossover chromatids has received information from the other at a given site. Such tetrads would arise for markers on either side of the DSB, as shown in h and i. The rarity of these tetrads at *ARG4* of yeast casts doubt on the concept that noncrossovers typically arise from cutting of the two Holliday junctions. The segments of hDNA indicated in the joint molecule (e) have not been experimentally confirmed (*e.g.*, Schwacha and Kleckner 1995).

on the same chromatid, a feature not predicted by the DSBR model. These observations raised two questions: first, in terms of Figure 1, how are opposite-sense resolutions effected while same-sense resolutions are avoided and, second, how does the joint molecule give rise to noncrossovers?

A second notable feature of the data of Gilbertson and Stahl (1996), reported previously by others (*e.g.*, Porter *et al.* 1993), was that a conspicuous fraction of the events appeared to be "one-sided"; *i.e.*, in about half of the tetrads that manifested conversion for the palindrome on one side of the DSB, the palindrome on the other side showed normal 4:4 segregation. These may have been tetrads in which resection from the DSB had been so lopsided that one of the palindromes was not included in the joint molecule. However, Sun *et al.* (1991) reported that 68% of the DNA that enjoyed a DSB was resected >440 bp. If resection lengths at *ARG4* are exponentially distributed, ~85% of the resections would be expected to reach the palindrome markers of Gilbertson and Stahl (1996; Figure 2). If resections have a minimal length, as they seem to have at another locus (Bishop *et al.* 1992), all of the resections might include the markers of Gilbertson and Stahl (1996). For simplicity, therefore, we assume that all resections proceeded beyond those two markers. If that assumption is correct, the canonical model predicts that both markers will give rise to 5:3 tetrads (half conversions, Figure 1) except when MMR intervenes. This prediction implies that MMR must account for 4:4 as well as 6:2 tetrads, *i.e.*, that both restoration- and conversion-type MMR occurs.

An additional feature of the data observed and addressed by Gilbertson and Stahl (1996) concerns a

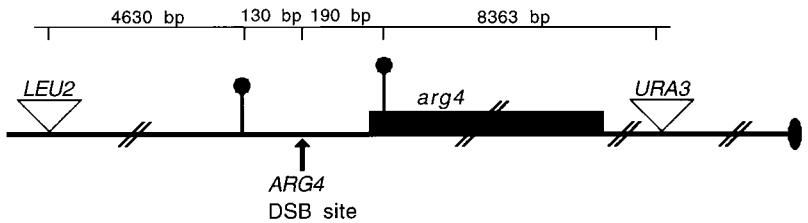

Figure 2.—The cross of Gilbertson and Stahl (1996). The segregating diploid was marked with small palindromes close to the DSB site on each side. The DSB site is in the promoter region of *ARG4*. The palindrome marker on the "right" side of the DSB site was in the 5′ end of the coding region of *ARG4*. These palindrome markers make relatively poorly repairable mismatches when in heteroduplex with their wild-type allele, allowing for the detection of half conversions (5:3 segregations) as well as full conversions (6:2 segregations). Strains carried nutritional markers flanking *ARG4*. One nutritional marker was 4.8 kb to the left of the *ARG4* DSB site; the other marker was 8.6 kb to the right of the DSB site.

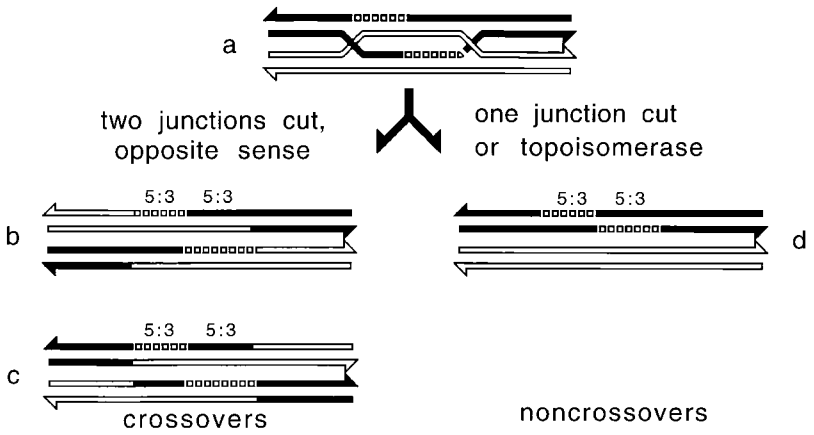

Figure 3.—Gilbertson and Stahl's (1996) modification of the canonical theory. The finding by Gilbertson and Stahl (1996) of the tetrads diagrammed in d drove home the point that most noncrossovers at *ARG4* are not the result of cutting two Holliday junctions and provided support for the idea of resolution mediated by topoisomerase or by the cutting of one junction followed by sliding of the other. In the absence of MMR and of any resection of the 3′-ended strands, as illustrated here, all aberrant segregations are half conversions (5:3 tetrads).

relationship between crossing over and MMR. Gilbertson and Stahl (1996) observed that, in addition to the relative shortage of doubly unrepaired heteroduplex among crossovers, there was a relative excess of 6:2 tetrads (full conversions) associated with crossing over. Specifically, for aberrant segregations presumed to arise by DSBs at the *ARG4* site, the following relations were seen: 5:3 on both sides of the DSB were crossovers 0.10 of the time; 5:3 on one side and 6:2 on the other were crossovers 0.46 of the time; 6:2 on both sides were crossovers 0.68 of the time. The apparent correlation between full conversion and crossing over was rationalized by Gilbertson and Stahl (1996) with the assumption that junction cutting occurs preferentially in crossovers and, as proposed previously by Alani *et al.* (1994) and by Schwacha and Kleckner (1995), triggers a late round of mismatch repair. Junction cutting creates ends, which, they presumed, stimulate MMR.

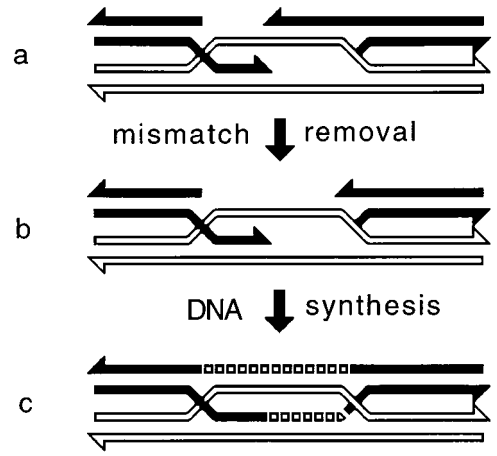

Figure 4.—Early MMR. Mismatch repair guided by the ends of the invading strands (a) can remove invading DNA, which is shown arbitrarily on the right side of the DSB (b). Synthesis then completes the joint molecule (c). While a marker on the left of the DSB will segregate 5:3 (half conversion), one on the right will segregate 6:2 (full conversion). MMR tied to invading ends leads to full conversions only, not to restorations (4:4 segregation). A half-converted marker on the left of the DSB would yield the forbidden aberrant 5:3 if resolution-producing noncrossovers were by cutting both junctions, but not if it were by topoisomerase or by cutting of only one junction.

Prompted by their data, Gilbertson and Stahl (1996) presented a slightly modified version of the canonical DSBR model. In Figure 3, their model is diagrammed for the instance in which MMR of heteroduplexes fails for both of the palindrome markers. Gilbertson and Stahl (1996) proposed that noncrossovers arose primarily by topoisomeric resolution of the joint molecules. They noted that cutting of one junction followed by sliding of the uncut junction to that unligated site would give the same products. Thus, crossovers, presumed by Gilbertson and Stahl (1996) to arise in the canonical manner, *i.e.*, f or g in Figure 1, suffer more cut junctions than do noncrossovers and therefore undergo more MMR than noncrossovers.

Our modification of this model is based on two proposals: (1) To account for the rarity of same-sense, but abundance of opposite-sense, resolutions of Holliday junctions (Figure 1), we propose that each junction has a structural asymmetry that biases which strands are cut; and (2) retaining the conventional assumption that strand ends not only stimulate MMR but direct it to occur on the discontinuous strand, we follow the suggestion of Alani *et al.* (1994) that MMR is often directed not by the discontinuities at the DSB site (Figure 4) but rather by cuts at the Holliday junctions (Figure 5). A relationship between MMR and crossing over is implicit in these proposals if crossovers, but not noncrossovers, are derived from joint molecules in which both junctions are cut. A further implication is that junction-directed MMR will result in either restoration of 4:4 segregation or full conversion (6:2 segregation), depending on the direction of junction-cutting and the location of the affected mismatch relative to the DSB. Restoration-type repair could account for Gilbertson and Stahl's (1996) one-sided events; it could also contribute to gradients of aberrant segregation frequencies (conversion gradients) such as those observed, for example, in the *HIS4* and *ARG4* genes.

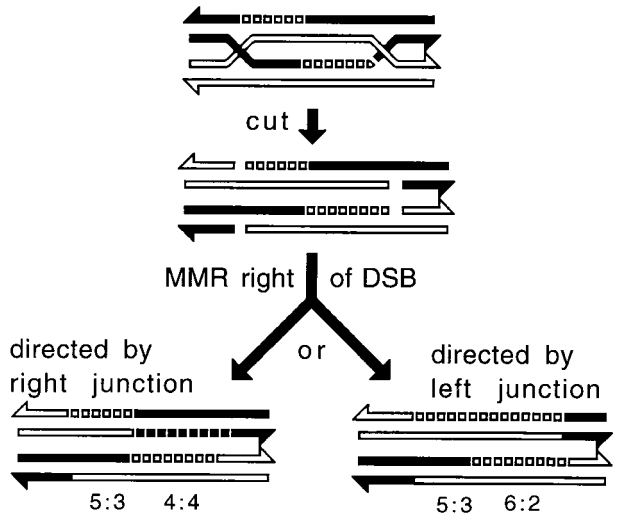

Figure 5.—Crossing over and late MMR guided by junction cutting. MMR keyed by cutting of the two Holliday junctions in the favored sense can result in restoration (4:4) of a marker on the same side of the DSB as a cut junction (left), or full conversion (6:2) of a marker on the other side of the DSB from the cut junction (right) as illustrated for the right-hand marker.

THE MODEL

Our primary modification of the DSBR model is forced by the first proposal, that a structural asymmetry dictates which strands of a Holliday junction are cut. The only apparent asymmetry in the canonical intermediate (Figure 1e) is the disposition of the newly synthesized DNA. At one junction, the new DNA is on a Crick strand, while at the other junction it is on a Watson strand. We propose that this new DNA, or its synthesis, dictates both Watson strands to be cut (or left uncut, depending on the direction of the bias) at one junction and both Crick strands to be cut (or not) at the other junction. Strict adherence to this rule will generate crossovers of either type f or type g in Figure 1, but not both. In an alternative scheme mentioned by Gilbertson and Stahl (1996), one of the junctions is cut at random, while a "machine" then constrains the cutting of the second junction to be opposite that of the first. This scheme predicts crossovers of type f and type g in equal frequencies.

The existence of a bias, as well as its sense, is implied by a feature of the Gilbertson and Stahl (1996) data. Those authors attempted to "locate the exchange" that unlinked the flanking markers. They did so in the manner proposed by Fogel *et al.* (1979), by asking whether the minority marker at a site segregating 5:3 had become unlinked from the flanking marker located on the same side of the recombination-initiating site. That tactic was devised at a time when the Aviemore model (Meselson and Radding 1975), in which a single Holliday junction represented a crossover, held sway, and it made some sense within that context. In the DSBR model, however, crossing over is the result of the combined mode of resolution of two Holliday junctions. In this context, therefore, linkage (or lack of linkage) between the minority marker at a site segregating 5:3 and a more distant marker on the same side of the DSB does not locate the exchange, but, instead, tells which strands are cut to give the required opposite-sense resolution.

Gilbertson and Stahl (1996) examined 5:3 segregants for the palindrome on the right side of the DSB and noted that, among crossovers, the minority marker tended to remain linked to its flanking marker on the right. The linkage was 84%, instead of the 50% expected from the hypothesis of unbiased cutting. Similarly, for tetrads 5:3 at the marker to the left of the DSB, among crossovers the minority marker tended to remain linked to its flanking marker on the left. This result indicates that the mode of resolution is indeed nonrandom and that the preferred mode involves the cutting of those chains that were newly synthesized at the junction—resolution f is favored over resolution g. Others observed a bias in the same direction (Fogel *et al.* 1979; White and Petes 1994; Hillers and Stahl 1999).

The identification of a bias in junction cutting allows us to explore the potential consequences of MMR triggered by junction cuts. In Figure 5, we illustrate MMR in the context of crossing over, with resolvase cutting in the favored orientation. The strand discontinuities introduced by resolvase determine the strands upon which excision will occur during MMR. Junction-directed MMR can result in either restoration or full conversion as illustrated for MMR occurring (arbitrarily) on one strand or the other of the upper duplex. Other patterns can arise if both duplexes are repaired or if MMR is triggered both by the DSB ("early repair"), as illustrated in Figure 4, and by junction cuts ("late repair"). Note that early repair alone generates no restorations and that late repair triggered by "unfavored" junction resolution (Figure 1g) can do so only for mismatches beyond the DSB. The assumption, adopted in Figures 5–9, that MMR occurs after junction resolution, implies a competition between cut junctions for the right to direct the repair of a given mismatch, making it likely that proximity of a mismatch to a junction increases the likelihood that its repair will be directed by that junction (see also calculations and discussion). Thus, among crossovers resolved in the "favored" mode, mismatches far from the DSB would be more likely to undergo restoration-type repair than conversion-type repair, while among crossovers resulting from "unfavored" resolution such mismatches would be more likely to undergo conversion-type repair.

If the observed relative paucity of noncrossovers among tetrads with full conversions reflects reduced opportunity for late MMR, noncrossovers must suffer fewer junction cuts, *i.e.*, one or none, than do crossovers, which suffer cuts at both junctions. We propose that noncrossovers arise from the resolvase cutting of one

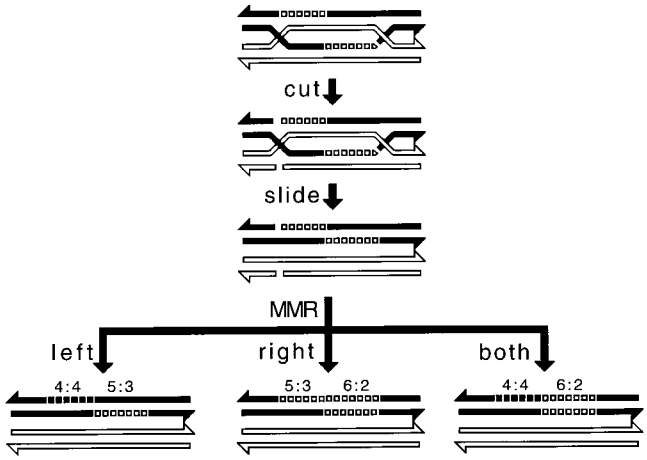

Figure 6.—The possible outcomes of late MMR in noncrossovers. MMR keyed by cutting of one Holliday junction, in the favored sense, can, in principle, have any of the following three outcomes: it can result in restoration (4:4) of a marker on the same side of the DSB as the cut junction (left), full conversion (6:2) of marker on the other side of the DSB from the cut junction (middle), or both restoration and full conversion (right).

Holliday junction accompanied by sliding of the other junction. Within the constraints of our model, the choice of single junction-cutting-with-sliding rather than topoisomerase is a forced move—the recovery of noncrossover tetrads with full conversion on one side of the DSB and normal 4:4 segregation on the other side argues for MMR potentiated by junction resolution. Possible segregation patterns resulting from such MMR directed by "favored" resolution of the junction on the left are illustrated in Figure 6, which arbitrarily allows both continuous and discontinuous repair tracts (see below).

The combination of location-dependent late repair and favored junction resolution suggests a mechanism for the restoration-conversion mix of repair proposed by Detloff *et al.* (1992) to account for the dependence of steep conversion gradients on efficient MMR. The authors proposed that the observed gradients represent an increasing likelihood for restoration-type repair with increasing distance of the mismatch from the DSB. Thus, to effect a gradient, efficient MMR must cause full conversion for mismatches close to the DSB while masking mismatches nearer the Holliday junction

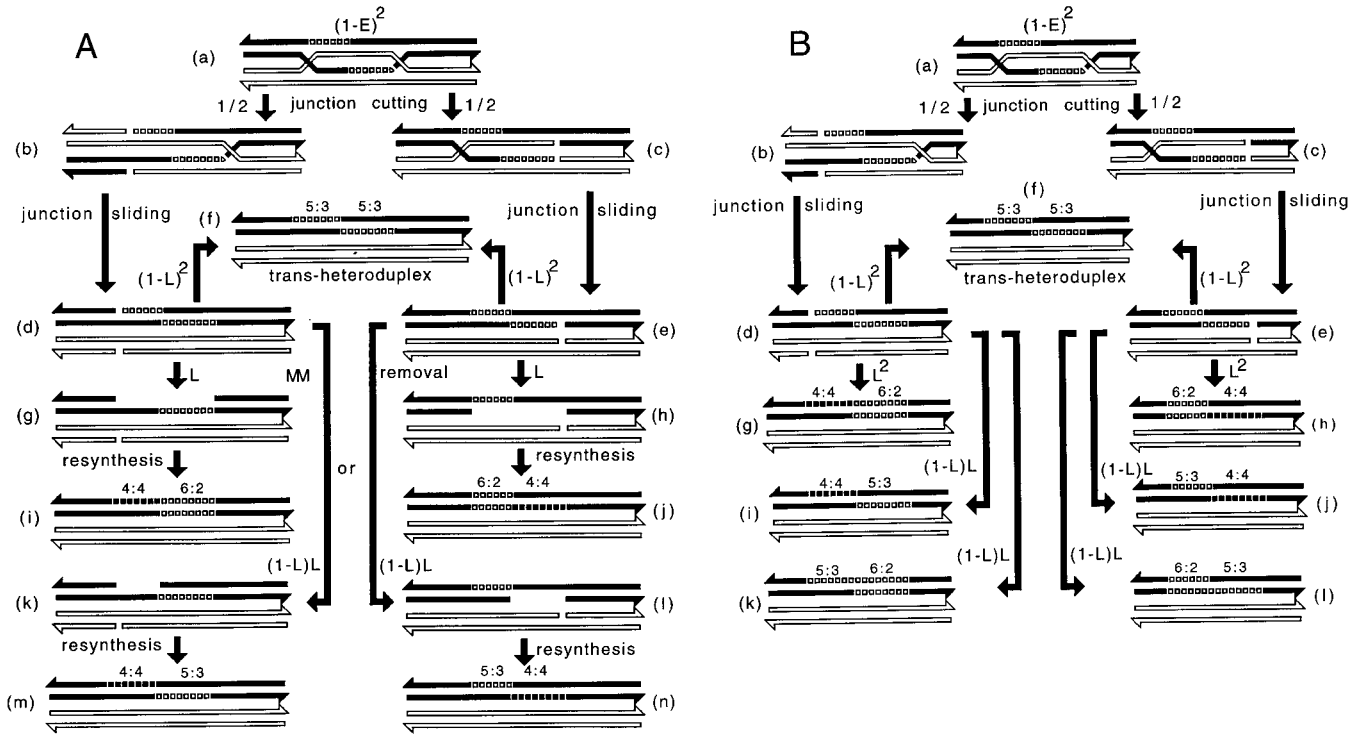

Figure 7.—Noncrossing over with MMR directed by resolvase nicks in the absence of early MMR. (A; variation 1) A joint molecule (a), with heteroduplex on both sides of the initiating DSB, can be cut at either junction with equal probability to give b or c. The cuts shown are the ones favored by the model, wherein resolvase action is guided by the newly synthesized DNA. The uncut junctions slide to the sites of the cut junctions to give d and e. With probability $(1 - L)^2$, MMR fails to operate at both heteroduplexes, yielding the *trans*-heteroduplex (f). With probability L, MMR operates on the mismatch farther from the strand end, removing DNA from the mismatch to the end, giving rise to g and h. Resynthesis gives the products i and j. With probability $(1 - L)L$, MMR fails for the site far from the end but succeeds for the site closer to the end, removing DNA from that site to the end (k and l). Resynthesis then gives the products m and n. (B; variation 2) a–f are as in A. In g and h the two mismatches have been independently repaired, with probability L^2. In i–l, one or the other has been repaired, with probability $(1 - L)L$.

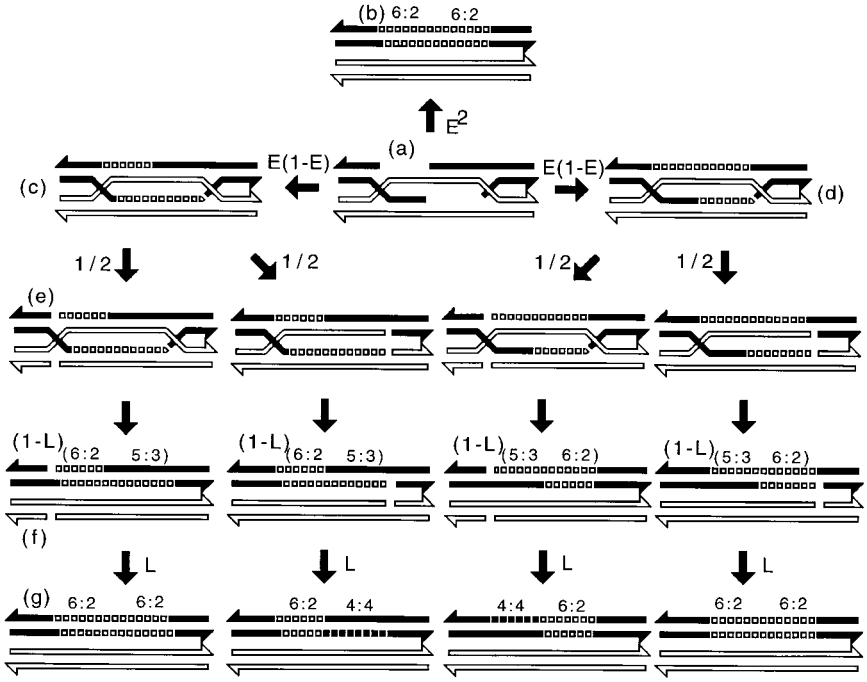

Figure 8.—Noncrossing over with MMR directed by resolvase nicks, following early MMR. When early MMR has repaired one or both mismatches, as diagrammed here, variations 1 and 2 are equivalent. The nascent joint molecule (a) can be repaired at both mismatches (probability E^2), giving product (b), or at one mismatch [probability $E(1 - E)$], giving joint molecules (c or d). These joint molecules can be cut by resolvase at either junction, giving intermediates (e) whose junctions slide to give unligated products (f). If MMR fails (probability $1 - L$), these intermediates are recovered as ligated products. If MMR succeeds (probability L), the products recovered are those in g.

through restoration-type repair. Defects in MMR would prevent half conversions from becoming undetectable restorations and would, consequently, result in longer, shallower gradients. We propose that, at the DSB site, MMR of well-repairable mismatches is directed exclusively from the DSB, resulting in full conversions and preempting restoration-type repair. With increasing distance from the DSB, mismatches would become more likely to escape early repair and would, therefore, become subject to junction-directed restoration-type repair. This hierarchical relationship between early and late repair would result in a conversion gradient. We envision that such a gradient would add to the effect conferred by heteroduplex rejection (Hillers and Stahl 1999).

CALCULATIONS

Adopting the first proposal outlined above, that Holliday junction resolvase preferentially cuts the pair of strands, one of whose members was newly synthesized in the neighborhood of the junction during formation of the joint molecule, we derive expectations for the distribution of tetrad types from the data of Gilbertson and Stahl (1996), based on the second proposal, that at least some of the discontinuities that trigger MMR must be created by Holliday junction resolvase. We outline the possible consequences of such late MMR as well as of the early MMR that can occur after the invasion step, before DNA synthesis completes the joint molecule. Expected frequencies of tetrad classes are expressed in terms of the probability of repair of a given poorly repairable mismatch by the early or late pathways. In assigning numerical values to these probabilities it is not our intention to find the best fit, but simply to ascertain (1) whether or not there is a set of values that allows the model to approximate the data and (2) whether such a set implies a substantial contribution of late MMR to total MMR of poorly repairable heteroduplexes as proposed in the model. Should there be such a set, we would consider the model a candidate for experimental test and refinement.

The predicted consequences of early and late MMR overlap but do not coincide. Early MMR produces only full conversions (6:2) and never restorations of 4:4 segregation (Figure 4). Late MMR, on the other hand, can generate restorations as well as full conversions. A mismatch on the same side of the DSB as the cut junction that directs the MMR results in restoration of normal segregation, while MMR for a mismatch on the far side of the DSB results in full conversion (Figure 5). When early MMR has acted at both marked sites, late MMR is preempted. When early MMR has acted at only one site, late MMR can act only at the other site. When early MMR has failed at both sites, late MMR can act at either or both sites.

In our calculations, we make the following simplifying assumptions: (1) All junction cutting is effected by cutting the two strands in one of which there is newly synthesized DNA near that junction; (2) all mismatches of Gilbertson and Stahl (1996) are equally correctable; (3) the palindromes of Gilbertson and Stahl (1996) are sufficiently close to each other that the difference in their distances from the two Holliday junctions is not a factor; (4) the marker excised by MMR is always the one that is *cis* to the strand end; (5) late MMR occurs after resolution of the joint molecule, which for noncrossovers means after sliding the uncut junction

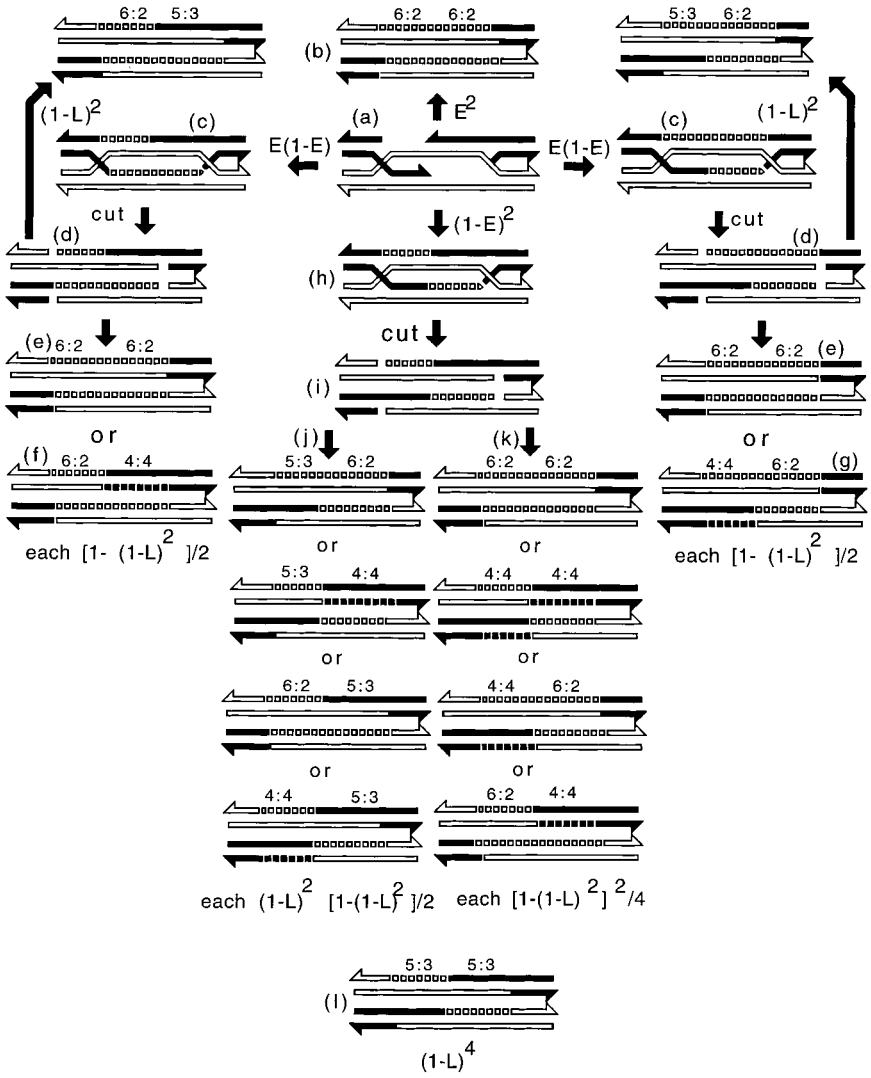

Figure 9.—Crossing over with or without early MMR. Variations 1 and 2 are equivalent. The nascent joint molecule (a) can undergo early repair at both mismatches (probability E^2), giving product (b), or at one mismatch [probability $E(1 - E)$], giving joint molecules (c). The joint molecules are cut in the favored crossover pattern (d). The resulting resolved duplexes can be ligated without MMR [probability $(1 - L)^2$], giving products (e), or their mismatches can be repaired from either cut junction to give f or g {probability of each is $[1 - (1 - L)^2]/2$}. If early MMR fails [probability $(1 - E)^2$], the joint molecule (h) is cut (in the favored pattern) to give a pair of duplexes, each member of which has a heteroduplex region and two strand interruptions that can direct MMR (i). MMR on one duplex only (j) has four possible outcomes, each with a probability $(1 - L)^2[1 - (1 - L)^2]/2$. MMR on both duplexes (k) has four possible outcomes, each with probability $[1 - (1 - L)^2]^2/4$. All opportunities for MMR may fail, giving type l with probability $(1 - L)^4$.

to the location of the cut junction [this assumption is forced by consideration that late MMR prior to junction sliding would result in aberrant 4:4 rather than normal 4:4 segregation (restoration)]; (6) in early MMR, corrections of the two mismatches are independent of each other; and (7) among crossovers, late MMR, too, occurs independently for each of the two mismatches, because repair is triggered by junction cuts on separate chromatids. Among noncrossovers, on the other hand, late MMR for the two mismatches is triggered by a single junction cut, which may or may not affect both mismatches. We therefore present our calculations for each possibility. In variation 1, we presume that MMR excises DNA between the site of either mismatch and the discontinuity created by Holliday junction resolution. Such a rule is suggested by the *in vitro* properties of the MMR system of *Escherichia coli* (Grilley *et al.* 1993). In variation 2, the removal need not be continuous. Such a possibility is suggested by the demonstration in yeast (Kirkpatrick *et al.* 1998) of tetrads in which a site segregating normally is flanked by sites segregating 5:3. Earlier data supporting the same view are summarized by Fogel *et al.* (1981). The difference in the two variations affects the formalizations of the models only for noncrossovers and only when neither mismatch has been repaired by early MMR.

Figures 7–9 are pictorial representations of the model, showing the steps in junction resolution and associated MMR with their associated probabilities. These figures formalize the assumptions and their algebraic consequences. Figures 7 and 8 describe noncrossovers in the absence and presence of early MMR, respectively. Figure 9 describes crossovers. E is the probability of early MMR of a given poorly repairable mismatch; L is the probability of late MMR of a given poorly repairable mismatch stimulated by a given junction cut. The pathways shown in Figures 7–9 permit us to calculate expectations for the relative frequencies of the tetrad classes observed by Gilbertson and Stahl (1996). The predictions of the two variations of the model are presented in Table 1.

To compare the calculated probabilities with ob-

TABLE 1

Predicted probabilities of the tetrad classes

	Crossovers	Noncrossovers
	Variation 1	
4:4 6:2	$E(1-E)^{1/2}[1-(1-L)^2] + (1-E)^2\,{}^1/_4[1-(1-L)^2]^2$	$E(1-E)^{L/2} + (1-E)^2\,{}^L/_2$
6:2 6:2	$E(1-E)[1-(1-L)^2] + E^2 + (1-E)^2\,{}^1/_4[1-(1-L)^2]^2$	$E^2 + E(1-E)L$
5:3 6:2	$E(1-E)(1-L)^2 + (1-E)^2\,{}^1/_2(1-L)^2[1-(1-L)^2]$	$E(1-E)(1-L)$
4:4 5:3	$(1-E)^2\,{}^1/_2(1-L)^2[1-(1-L)^2]$	$(1-E)^2(1-L)^{L/2}$
6:2 5:3	$E(1-E)(1-L)^2 + (1-E)^2\,{}^1/_2(1-L)^2[1-(1-L)^2]$	$E(1-E)(1-L)$
5:3 5:3	$(1-E)^2(1-L)^4$	$(1-E)^2(1-L)^2$
	Variation 2	
4:4 6:2	As for variation 1	$E(1-E)^{L/2} + (1-E)^2\,{}^{L^2}/_2$
6:2 6:2	As for variation 1	$E^2 + E(1-E)L$
5:3 6:2	As for variation 1	$E(1-E)(1-L) + (1-E)^2(1-L)^{L/2}$
4:4 5:3	As for variation 1	$(1-E)^2(1-L)^{L/2}$
6:2 5:3	As for variation 1	$E(1-E)(1-L) + (1-E)^2(1-L)^{L/2}$
5:3 5:3	As for variation 1	$(1-E)^2(1-L)^2$

served numbers (Table 2), we normalized the calculated probabilities to account for the fact that Gilbertson and Stahl (1996) enumerated only those tetrads in which segregation at the right palindrome was aberrant. The normalized probabilities were then multiplied by the observed total number of noncrossovers or of crossovers, respectively, to get the expected numbers for each observed tetrad class. The relative rarity of tetrads that manifest full conversion simultaneously for both of the palindrome markers suggests that late MMR is more frequent than early MMR, and we see that major features of the data of Gilbertson and Stahl (1996) are accommodated when $L = 0.6$ and E lies between 0 and 0.1. According to our model, these small E values characterize only poorly repairable mismatches. Well-repairable mismatches close to the DSB typically become full convertants via early repair.

The observed values for 4:4, 6:2 and for 5:3, 6:2 noncrossover tetrads are bracketed by the predictions of variations 1 and 2, suggesting that they are generated by a mix of the two variations.

In comparing the model with the data, we ignored the fact that a fraction of the crossovers enumerated by Gilbertson and Stahl (1996) are incidental to the conversions at the palindrome sites. We also ignored the evidence that some (~16%) of the crossover resolutions break our assumed rule for junction cutting. Additionally, by writing a model with symmetric probabilities,

TABLE 2

Calculated frequencies compared with frequencies from Gilbertson and Stahl's (1996) Table 3

	Noncrossovers			Crossovers	
		Calculated			
	Observed	Var. 1	Var. 2	Observed	Calculated
$E = 0.1$; $L = 0.6$					
4:4, 6:2	44	66.1	36.7	22	29.8
6:2, 6:2	7	15.8	13.6	15	37.9
(5:3, 6:2)[a]	38	17.6	56.4	33	22.8
4:4, 5:3	40	23.9	20.6	30	9.0
5:3, 5:3	26	31.6	27.6	3	3.5
Σ	155	155	155	103	103
$E = 0$; $L = 0.6$					
4:4, 6:2	44	80.2	39.8	22	31.3
6:2, 6:2	7	0	0.0	15	31.3
(5:3, 6:2)[a]	38	0	53.2	33	23.8
4:4, 5:3	40	32.1	26.6	30	11.9
5:3, 5:3	26	42.8	35.4	3	4.5
Σ	155	155	155	103	103

[a] (5:3, 6:2) combines the symmetric classes 5:3, 6:2 and 6:2, 5:3.

we have ignored asymmetries reported by Gilbertson and Stahl (1996): (1) MMR more often corrected in favor of the wild-type allele of the palindrome markers; (2) mismatches for one palindrome appeared to be corrected more often than mismatches for the other palindrome; and (3) initiations were more often on one chromosome than the other. These asymmetries do not bear directly on the issues examined here. Furthermore, our calculations, which average out such asymmetries, are compared with combined data from two types of crosses (one parent carrying both palindromes or each parent carrying one palindrome), which also average out asymmetries.

Our calculations led to underprediction of the 4:4, 5:3 class. The discrepancies are in the same direction for both noncrossovers and crossovers and for variations 1 and 2, so that no mixture of the two variations will relieve the problem. Heteroduplex rejection (see Hillers and Stahl 1999) is likely to contribute to the 4:4 segregant class. Also, our assumption that resection is always greater than the 130-bp distance from the DSB site to the left palindrome may be wrong, so that some of the 4:4 segregants are truly "no events." Alternatively, we may note that in both of the variations of the model the (4:4, 5:3) tetrads arise when the site nearer to the relevant cut junction (on the left) is repaired without involvement of the more distant site. That consideration suggests that our assumption that each marker triggers MMR without regard to its position relative to the other marker and/or distance from the points of junction resolution was too simple.

DISCUSSION

Gilbertson and Stahl (1996) suggested that "one-sided events" (aberrant segregation on one side of the DSB and 4:4 segregation on the other) reflect early, reversible interactions of the cut chromatid with its sister. The considerations presented here do not rule out such possibilities but do offer a testable alternative. If one-sided events represent mismatches rendered undetectable by restoration-type MMR, these mismatches should become detectable as an increase in the frequency of aberrant segregation when MMR efficiency is reduced. Experiments by other workers failed to demonstrate a significant increase in aberrant segregation frequency for well-repairable markers near the high end of the conversion gradients in *ARG4* and/or *HIS4* when MMR was reduced by a mutation in the *MSH2* gene. For markers far from the DSB site, however, aberrant segregation frequencies were increased by mutations in genes governing MMR (Alani *et al.* 1994; Hunter and Borts 1997). These data are compatible with the proposal that poorly repairable mismatches near the DSB, and well- or poorly repairable mismatches far from the DSB, escape "early" conversion-type MMR directed from the DSB and thereby become subject to restoration-type MMR directed by junction cuts.

The study by Hillers and Stahl (1999) permits further tests of our model. First, the prediction that junctions should be cut predominantly in the favored direction is supported by the data—among crossover tetrads, 57–60% had type-f resolution at *HIS4* (Table 4, Hillers and Stahl 1999). The weakness of the bias as compared with that at *ARG4* implies a relatively high frequency of random junction cutting at *HIS4*, offering the opportunity to test other aspects of the model. For example, increased randomness in junction cutting should cause increased frequencies in "same sense" resolutions (as in Figure 1i) as well as in type g resolutions. In fact, Hillers and Stahl (1999) found ~40 tetrads of type i whereas Gilbertson and Stahl (1996) found none in an *ARG4* analysis approximately as large. (Note that, if unfavored resolutions result from the loss of guiding signals in the meiotic cell, the model predicts approximately equal numbers of type g and type i resolutions. The observed inequality suggests that additional factors are involved in junction resolution.)

A relative excess of tetrads of types g and i is predicted to result in a shallower conversion gradient, possibly contributing to the relative shallowness of the gradient at *HIS4* as compared with *ARG4* (Detloff *et al.* 1992). The data of Hillers and Stahl (1999) allow us to determine the distribution of conversion-type repair for DSB-distant mismatches among resolution types. Hillers and Stahl's (1999) Table 4 shows that, among half conversions at the 5′ marker, the frequency of full conversions for a well-repairable 3′ marker was $35/82 = 0.43$ for combined tetrads of type g + i, in contrast to $22/150 = 0.15$ for tetrads of type j + h, and $6/73 = 0.08$ for tetrads of type f. These data and similar, though scant, data for a poorly repairable marker support our suggestion that, by their penchant for conversion-type repair, tetrads of types g + i diminish the slope of the conversion gradient.

Our model predicts the following: type i resolutions direct full conversions only; type g resolutions direct predominantly full conversions, triggering restorations only from junction cuts on the far side of the DSB; type j resolutions direct restorations and conversions in approximately equal numbers; type f resolutions direct predominantly restorations, triggering full conversions only for mismatches beyond the DSB; and type h resolutions can direct restorations only. The observation that the frequency of conversion-type repair is lowest for resolution type f, intermediate for type j + h, and highest for type g + i supports half of this prediction; support for the other half calls for a reverse correlation with respect to restoration-type repair. To test this latter prediction, we examined the resolution types identified in Hillers and Stahl's (1999) Table 4 for evidence of restoration-type repair.

Restoration-type repair, as well as heteroduplex rejec-

TABLE 3

Resolution-dependent increases in aberrant segregation frequency in response to reduced MMR

Resolution type	Aberrant segregation frequency[a]		Factor increase
	Full MMR[b]	Reduced MMR[c]	
j + h	22/150	79/150	3.6
f	6/73	39/66	7.2
glll	24/31	33/36	1.2
illl	9/12	6/6	1.3
(g + i + g/i)lll	35/45	39/43	1.2
gll + glll	24/48	32/43	1.5
ill + illl	9/24	6/10	1.6
all i + all g	35/82	38/56	1.6

[a] Number of tetrads with half conversion for a DSB-proximal marker (*his4-IR9*) and full or half conversion for a DSB-distal marker (either *his4-713* or *his4-3133*) divided by the total number of tetrads with half conversion for the DSB-proximal marker. Values are from Table 4 of Hillers and Stahl (1999).

[b] Crosses KY48 + KY49 (Hillers and Stahl 1999).

[c] Crosses KY51 + KY52 (Hillers and Stahl 1999).

tion, can manifest itself indirectly as an increase in aberrant segregation events in response to reduced MMR. The contribution to this increase from heteroduplex rejection (assumed to occur independently of subsequent junction resolution) should be identifiable. In tetrads of type gIII + iIII, which by definition did not undergo heteroduplex rejection, the effect of reduced MMR could reflect only the minimal restoration-type repair expected for tetrads of type g, whereas in the full class of type g (or g + i) tetrads such an effect should represent both heteroduplex rejection and restoration-type repair. It follows that any increase in the effect of reduced MMR in tetrads of types j + h and f over tetrads of type g (or g + i) may be attributed to the relatively greater incidence of restoration-type repair predicted for tetrads of types j + h and f. Table 3 confirms a minimal incidence of restoration-type repair in tetrads of type g + i, a greater incidence of heteroduplex rejection (last three rows), presumably occurring in all tetrad types, and a progressively larger incidence of restoration-type repair in tetrad types j + h and f, respectively, as predicted. The negligible evidence for restoration-type repair in tetrads of type gIII implies that, for the crossovers in these experiments, proximity to the directing junction is the primary factor governing the type of repair (full conversion *vs.* restoration) of DSB-distal mismatches.

Thus, the results of Hillers and Stahl (1999) support the crucial tenets of the model: (1) junction cutting directs mismatch repair; (2) junctions cut in the favored sense direct restoration-type repair, while junctions cut in the unfavored sense direct conversion-type repair; and (3) the relationship between junction resolution and mismatch repair holds for all resolution types (Figure 1), suggesting that noncrossovers as well as crossovers are derived from the joint molecule intermediate. Finally, the correlation between the strength of the resolution bias and the steepness of the conversion gradient, together with the correlation between favored resolutions and restoration-type repair, indicate a major role for restoration-type repair in the formation of the conversion gradient.

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