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Meiotic Recombination in Drosophila *Msh6* Mutants Yields Discontinuous Gene Conversion Tracts

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ABSTRACT

Crossovers (COs) generated through meiotic recombination are important for the correct segregation of homologous chromosomes during meiosis. Several models describing the molecular mechanism of meiotic recombination have been proposed. These models differ in the arrangement of heteroduplex DNA (hDNA) in recombination intermediates. Heterologies in hDNA are usually repaired prior to the recovery of recombination products, thereby obscuring information about the arrangement of hDNA. To examine hDNA in meiotic recombination in *Drosophila melanogaster*, we sought to block hDNA repair by conducting recombination assays in a mutant defective in mismatch repair (MMR). We generated mutations in the MMR gene *Msh6* and analyzed recombination between highly polymorphic homologous chromosomes. We found that hDNA often goes unrepaired during meiotic recombination in an *Msh6* mutant, leading to high levels of postmeiotic segregation; however, hDNA and gene conversion tracts are frequently discontinuous, with multiple transitions between gene conversion, restoration, and unrepaired hDNA. We suggest that these discontinuities reflect the activity of a short-patch repair system that operates when canonical MMR is defective.

CROSSOVERS (COs) generated through meiotic recombination are essential to the correct segregation of chromosomes during meiotic divisions in most eukaryotes. An understanding of the events required to generate COs is central to the understanding of this crucial phenomenon. Several models of meiotic recombination have been proposed to describe the molecular steps required to generate COs. These models must account not only for CO formation, but also for the formation of noncrossovers (NCOs) and the association of gene conversion (GC) with both COs and NCOs.

More than 40 years ago, Robin Holliday proposed a model for meiotic recombination to account for these observed phenomena (Holliday 1964). One prominent feature of this model is the suggestion that GC associated with both COs and NCOs is generated by the formation and repair of heteroduplex DNA (hDNA), DNA in which each strand of the duplex is derived from a different parental chromosome. Although several other models for meiotic recombination have been proposed over the years, invoking different initiating lesions and different recombination intermediates, formation and repair of hDNA as a mechanism for GC has remained a constant (Meselson and Radding 1975; Szostak *et al.* 1983).

Experimental evidence supports models in which the formation and repair of hDNA are important features of meiotic recombination. Physical characterization of recombination at a meiotic hotspot in *Saccharomyces cerevisiae* has revealed hDNA in recombination intermediates (Allers and Lichten 2001). Additionally, the products of meiotic recombination sometimes exhibit postmeiotic segregation (PMS), when the two parental alleles segregate from one another at the first postmeiotic mitosis. This is thought to arise from unrepaired heterologies in hDNA, since PMS occurs most frequently in mutants that abolish mismatch repair (MMR) (reviewed in Borts *et al.* 2000) or for heterologies that are not repaired efficiently, such as short palindromic insertions (Nag *et al.* 1989).

Although the formation and repair of hDNA is a common feature of models of meiotic recombination, the structure and arrangement of hDNA present in the proposed recombination intermediates and products differ in different models. Investigation of hDNA present in the PMS products of meiotic recombination in *S. cerevisiae* has provided insights into the mechanism of CO formation (Foss *et al.* 1999; Merker *et al.* 2003; Hoffmann and Borts 2005; Hoffmann *et al.* 2005),

We dedicate this article to the memory of Jennifer Leigh Edwards Zartman, who made the first attempt to generate a deletion in *Msh6*.

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but the degree to which details of the structure and arrangement of hDNA can be determined through these experiments is limited. Detection of hDNA requires the presence of heterologies between homologous chromosomes, and it has been shown that high levels of heterology in *S. cerevisiae* decrease the frequency of recombination (Borts and Haber 1987). Levels of heterology ranging from ~0.05 to ~0.3% have been used in experiments in *S. cerevisiae*, but the highest levels have included, at most, only three polymorphisms (Borts and Haber 1987; Judd and Petes 1988; Symington and Petes 1988; Gilbertson and Stahl 1996; Merker *et al.* 2003; Jessop *et al.* 2005).

In *Drosophila melanogaster*, high levels of heterology (~0.5%) do not affect the frequency of meiotic recombination (Hilliker *et al.* 1991). We have previously studied recombination between two highly polymorphic chromosomes (33 polymorphisms across 7420 bp, ~0.45%) in Drosophila and have used the multiple heterologies to determine GC tract lengths with high resolution (Blanton *et al.* 2005). The study of unrepaired hDNA in the PMS products of meiotic recombination in Drosophila provides an opportunity to gain insight into the structure and arrangement of hDNA at a high resolution. In *S. cerevisiae*, even well-repaired markers, such as base–base mismatches or small insertion/deletion loops, sometimes go unrepaired, resulting in PMS (Fogel *et al.* 1981). In contrast, PMS is exceedingly rare in Drosophila (Chovnick *et al.* 1971), even though the same types of heterologies should be present in hDNA. To analyze the structure of hDNA, thereby gaining insight into the mechanism of meiotic recombination in Drosophila, we sought to decrease repair by removing the canonical MMR pathway.

The MutS and MutL proteins are central components of MMR in *Escherichia coli* (reviewed in Modrich and Lahue 1996); canonical MMR in eukaryotes involves several homologs of MutS (Msh proteins) and MutL (Mlh/Pms proteins) (reviewed in Kunkel and Erie 2005). The Drosophila genome has two genes encoding Msh proteins, *spel1* (encodes ortholog of Msh2) and *Msh6*, and two genes encoding MutL homologs, *Mlh1* and *Pms2* (reviewed in Sekelsky *et al.* 2000). We generated deletions that remove most of the coding sequence of *Msh6*. We report here that mutation of Drosophila *Msh6* greatly increases the incidence of PMS among both CO and NCO products of meiotic recombination. This suggests that hDNA is a common feature of meiotic recombination intermediates in Drosophila and that MMR homologs are important for the repair of this hDNA. Surprisingly, tracts of GC and unrepaired hDNA from *Msh6* mutants are frequently discontinuous, a phenomenon that we did not observe among wild-type CO and NCO products (S. J. Radford and J. Sekelsky, unpublished results; Blanton *et al.* 2005; Radford *et al.* 2007, this issue). We propose that, as has been suggested in *S. cerevisiae* (Coic *et al.* 2000), in the absence of canonical MMR, mismatches can be repaired through a process that allows closely spaced mismatches to be repaired or left unrepaired, independent of one another. While this short-patch repair system obscures much of the information provided by the structure and arrangement of hDNA, analysis of the remaining unrepaired hDNA provides insights into the mechanism of meiotic recombination in Drosophila.

MATERIALS AND METHODS

Recovery of recombination events within the *ry* gene: Thirty to 40 females of the genotype *y; $Msh6^{10}$ ry^{531} cv-c/$Msh6^{68}$ kar ry^{606}* were crossed to 10 to 15 males of the genotype *y/Y, Dp(1;Y)y^{+}; kar ry^{506} cv-c*. Crosses were set up in bottles containing 25 ml of standard food medium and placed at 25°. After 3 days, adults were transferred to fresh media to establish a second brood, and purine was added to the first brood bottles. We used 0.75 ml of 0.18% (w/v) purine in water, which is the lowest level that effectively kills the majority of *ry*– progeny (Radford *et al.* 2007), maximizing the survival of *ry*–//*ry*+ mosaics. One of every 25 bottles was left untreated and adult progeny were counted to estimate the number of larvae screened. Recombinant progeny were scored for flanking visible markers (karmoisin and crossveinless) to determine whether a CO or an NCO event had occurred.

Detection of gene conversion and PMS tracts: Recombinant progeny were mated to *kar ry^{506} cv-c* flies of the opposite sex to test for mosaicism via germline transmission. The recombinant fly was then homogenized in buffer containing proteinase K, as described (Gloor *et al.* 1993), to recover DNA for PCR. GC and hDNA tracts were analyzed by a combination of allele-specific and non-allele-specific PCR and sequencing of PCR products in bulk.

Allele-specific PCR primers were designed to specifically amplify each allele at each of several polymorphic sites (Figure 1A). Primers were designed such that the 3′ end corresponded to the allele-specific nucleotide, and an additional mismatch was engineered two or three nucleotides before the 3′ end to increase specificity. Annealing temperature and magnesium concentration were optimized for each primer pair. A list of polymorphic sites is given in supplemental data (supplemental Table S1 at http://www.genetics.org/supplemental/), along with allele-specific primer sequences and conditions (supplemental Table S2 at http://www.genetics.org/supplemental/). To prevent false-positive amplification primers were tested against positive and negative control fly preps with each use. Amplification with both allele-specific primers for a given site was taken as evidence for PMS at that site. Allele-specific PCR products were sequenced to determine the length and arrangement of hDNA tracts (Figure 1B). PMS was also detected by amplifying fragments with non-allele-specific primers, sequencing the bulk product, and examining the chromatogram for double peaks at polymorphic sites (Figure 1B).

Recombinants were recovered in *trans* to the ry^{506} chromosome, which has a deletion beginning at base pair 1398 and extending 3.8 kb to the right; this deletion does not include the region around ry^{606}, which is at −468. The ry^{506} deletion was induced on the same parental chromosome as ry^{531}, so both chromosomes have the same sequence differences relative to ry^{606}. For sequences to the right of ry^{606}, we analyzed PCR products where at least one primer was within the region deleted in ry^{506}, to ensure that products were derived from the recombinant chromosome. For regions to the left of ry^{606}, allele-specific PCR was done using a primer specific to the ry^{606}

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chromosome that was outside the region of GC or hDNA, as determined by sequencing.

Statistical comparisons: Mean GC tract lengths and statistical comparisons were calculated as described previously (Blanton *et al.* 2005). PMS frequency comparisons were made using Fisher's exact test with two-tailed *P*-values, computed by Instat 3.05 (GraphPad Software). Comparison of crossover distributions in Table 2 was done by two-tailed *G*-test.

RESULTS

Deletion of *Msh6* coding sequence by *P* excision: In yeast and mammalian systems, MMR is carried out by two functional heterodimers of MutS homologs, MutSα (Msh2 and Msh6) and MutSβ (Msh2 and Msh3) (reviewed in Kunkel and Erie 2005). Consequently, mutations in *MSH2* and *MSH6* do not elicit equivalent phenotypes in these organisms. The Drosophila and *Caenorhabditis elegans* genomes encode orthologs of Msh2 (SPEL1 in Drosophila) and Msh6, but not Msh3, suggesting that only MutSα is present in these organisms (Sekelsky *et al.* 2000; Denver *et al.* 2005). In support of this proposal, *C. elegans msh-2* and *msh-6* mutants have identical phenotypes with regard to mutation accumulation (Denver *et al.* 2005).

Flores and Engels constructed a synthetic deletion in Drosophila of *spel1* by combining overlapping deletions in *trans*, together with a transgene to replace a second gene contained in the region of overlap between the deletions (Flores and Engels 1999). These mutants were shown to exhibit increased microsatellite instability (Flores and Engels 1999), a common feature of MMR mutants in other species, suggesting that MMR function is conserved in Drosophila. On the basis of the results from *C. elegans*, we hypothesize that SPEL1 and MSH6 are equivalently required for MMR in Drosophila. Because *Msh6* is on chromosome *3*, the same chromosome as the locus used for our recombination assay (Blanton *et al.* 2005), it is technically easier to use mutations in this gene for characterization of unrepaired hDNA in the products of meiotic recombination in Drosophila.

We obtained a Drosophila stock in which a *P* element was inserted 43 bp upstream of the *Msh6* coding sequence and used it to generate deletions through excision of the *P* element. Mutations in *mus309* increase the recovery of flanking deletions following *P*-element excision (Adams *et al.* 2003; McVey *et al.* 2004b), so we used a PCR-based strategy to screen for deletions into *Msh6* coding sequence following excision in both wild-type and *mus309* mutant backgrounds. Of 153 *P*-element excisions from wild type, 1 (0.6%) had a deletion into *Msh6* coding sequence. In contrast, of 31 excisions from *mus309* mutants, 4 (13%) had a deletion into *Msh6* sequence, confirming that conducting excision in a *mus309* mutant background is an effective method for increasing the recovery of deletion mutants from *P*-element excision screens.

Through PCR and sequencing, we determined the breakpoints of four of five *Msh6* deletions. In all four cases, the deletion was in one direction from the *P*-element insertion. The single deletion obtained from wild type ($Msh6^{11}$) removed ∼850 bp of the 3.8-kb coding sequence and retained ∼160 bp of *P*-element sequence. The three mapped deletions obtained from *mus309* mutants were larger: $Msh6^{10}$, $Msh6^{59}$, and $Msh6^{68}$ remove 2.2 kb (+2.0 kb of *P* element), 3.8 kb (+12 bp of *P* element), and 2.8 kb (+130 bp of *P* element) of coding sequence, respectively. This suggests that an excision screen performed in a *mus309* mutant background not only increases the frequency of deletion, but also increases recovery of larger deletions.

To minimize the effects of other mutations that may have arisen during *P*-element excision, the experiments described below were performed in females heteroallelic for $Msh6^{10}$ and $Msh6^{68}$ (the *mus309* mutation was crossed off of each chromosome when *ry* alleles were crossed on). These alleles remove most of the coding sequence, so this genotype should represent a complete absence of *Msh6* activity.

Recovery of COs and NCOs is increased in *Msh6* mutants: Several MutS and MutL homologs are required for the generation of COs in *S. cerevisiae*, including Msh4, Msh5, Mlh1, and Mlh3 (Hunter and Borts 1997; Wang *et al.* 1999; Borner *et al.* 2004; Guillon *et al.* 2005). To determine whether Drosophila MSH6 has a role in CO formation, we recovered meiotic recombination events within the *rosy* (*ry*) locus, using a procedure developed by Chovnick and colleagues (Chovnick *et al.* 1970, 1971). The *ry* gene encodes xanthine dehydrogenase (XDH), which is required both for the metabolism of purine and for normal eye pigmentation. Females *trans*-heterozygous for ry^{531} and ry^{606}, point mutations separated by 3.8 kb (Figure 2), were crossed to males homozygous for ry^{506}, which deletes much of the gene. Rare rosy+ recombinants were selected by treating the larvae with purine; flanking markers were used to distinguish COs from NCOs (see materials and methods for details). Because an oocyte receives only one of the two chromatids involved in the recombination event (the other segregates into a polar body nucleus), we cannot determine whether the COs we recover have associated tracts of GC. NCOs, however, are recovered only when a GC (or PMS) tract spans one of the two *ry* mutations. Hence, we use the term "CO" to mean crossovers with or without gene conversion and the term "NCO" to mean gene conversion of a *ry* mutation (or PMS at that site) without an associated crossover.

We previously reported the results of an analysis of recombination events in wild-type females, in which we screened 2.3 million larvae and recovered 81 COs and 31 NCOs (Blanton *et al.* 2005). We have screened an additional 1.4 million larvae from wild type and recovered 31 COs and 22 NCOs (Table 1) (Radford *et al.* 2007). We screened 1.8 million larvae from *Msh6*

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TABLE 1

Intragenic recombination in wild-type and *Msh6* mutants

Genotype	Progeny screened	Crossovers		Noncrossovers	
		n (frequency)	PMS	*n* (frequency)	PMS
Wild type[a]	3,710,000	112 (3.0×10^{-5})	0	53 (1.4×10^{-5})	0
Msh6	1,775,000	67 (3.8×10^{-5})	14[b]	42 (2.4×10^{-5})	23[b]

[a] Includes data from Blanton *et al.* (2005) and Radford *et al.* (2007).
[b] PCR failed for DNA preps from one CO and two NCOs, so the frequency of PMS was 21% among COs (14 of 66) and 58% among NCOs (23 of 40).

mutants and recovered 67 COs and 44 NCOs, a 25% rate increase over wild type in COs and a 65% rate increase in NCOs (Table 1). These results show that *Msh6* is not required for the generation of COs.

Increased recovery of rosy+ recombinants in an MMR mutant is not unexpected. If one of the *ry* point mutations is included in hDNA during recombination, MMR may either restore the *ry*– sequence or convert it to *ry*+ sequence; flies carrying conversions will survive purine selection, but those receiving a chromatid that underwent restoration repair will not be recovered. If repair does not occur, as expected in an MMR mutant, the oocyte will receive a chromatid in which one strand is *ry*+ and the other is *ry*–, resulting in a *ry*+//*ry*– mosaic larva. Because XDH is secreted and diffuses throughout the developing larva, many *ry*+//*ry*– mosaics survive purine treatment, developing into mosaic adults that are rosy+ in eye color (Romans 1980). Thus, loss of MMR allows us to recover some events that would have been lost otherwise.

In *S. cerevisiae*, recombination is initiated at hotspots, which are usually in promoter regions (Gerton *et al.* 2000). In contrast, recombination at *ry* is thought to initiate and terminate throughout the gene (Clark *et al.* 1988). Hence, hDNA may include either mutation (ry^{606} is 1.2 kb 3′ of the transcription start site; ry^{531} is an additional 3.8 kb downstream, 0.5 kb from the 3′ end of the gene) or may be entirely between the mutations. In wild-type flies, we recover NCOs that include GC of either mutation, but in *Msh6* we can also recover those that would have been repaired to restore the mutant allele, since this will result in *ry*+//*ry*– mosaics. The frequency of recovery of NCOs was increased by 65% in the *Msh6* mutant, relative to wild type. This increase is consistent with MSH6 having a role in meiotic MMR and suggests that restoration repair often occurs in wild-type meiosis.

We recover COs that occur anywhere between the *ry* mutations so as to generate a *ry*+ chromosome. If a crossover includes hDNA spanning one of the mutant sites, repair of the hDNA to the mutant allele will prevent us from recovering the CO. As with NCOs, however, loss of MMR will allow us to recover these COs. Although we do not know the mean length of hDNA tracts associated with COs, the mean GC tract length among NCOs is 441 bp (Blanton *et al.* 2005), which is about one-ninth of the distance between the sites. Thus, it is probably a minority of COs that are lost due to repair of a mutant site, but are recovered in the *Msh6* mutant. In agreement with this prediction, the elevation in recovery of rosy+ recombinants was only 25% for COs (Table 1).

Both COs and NCOs from *Msh6* mutants exhibit PMS: To directly assess the role of MSH6 in hDNA repair, we examined the incidence of PMS among recombination events. Previously, PMS was detected in Drosophila by observing mosaicism in the germline and staining fly sections for the mosaic expression of XDH activity (Carpenter 1982); however, these methods can detect only PMS of the *ry* point mutations. To investigate fully the structure and arrangement of hDNA, it is necessary to detect PMS of both the *ry* point mutations and the surrounding silent polymorphisms. To accomplish this, we developed molecular assays for the detection of PMS.

We used one genetic assay and two molecular assays to detect PMS. First, we mated the rosy+ recombinants to ry^{506} partners to look for evidence of mosaicism in the germline. The Drosophila germline is set aside from a few nuclei out of several hundred present early in embryonic development. In a *ry*+//*ry*– mosaic, these germline cells may include only *ry*+, only *ry*–, or both *ry*+ and *ry*– maternal chromosomes; the latter two cases are definitive evidence for mosaicism. Among the 88 ry+ recombinants from *Msh6* mutants that produced progeny, two transmitted only *ry*– chromosomes, and none transmitted both *ry*+ and *ry*– maternal chromosomes. This is a large underestimate of the frequency of PMS, because this assay detects only a subset of *ry*+//*ry*– mosaics (Carpenter 1982) and can detect mosaicism only at the sites of the ry^{531} and ry^{606} mutations, not at sites of silent polymorphisms.

We used allele-specific PCR to detect PMS at both the *ry* point mutation sites and the surrounding polymorphisms (Figure 1A). Using allele-specific PCR for the point mutation sites, we detected 19 instances of PMS. This included 6 flies that did not produce progeny, 11 that transmitted only *ry*+ through the germline, and

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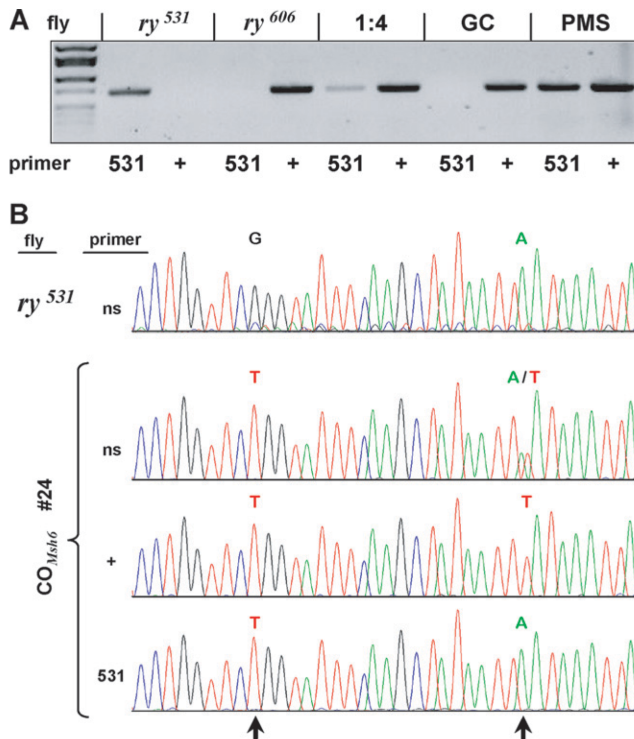

FIGURE 1.—Detection of PMS by allele-specific PCR and sequencing. (A) Allele-specific PCR. The image is from an agarose gel stained with ethidium bromide. DNA size markers were loaded in the leftmost lane. Other lanes contain products from PCR amplification of single-fly DNA preps. The sources of DNA were (left to right) a ry^{531}/ry^{506} fly, a ry^{606}/ry^{506} fly, a 1:4 mixture of ry^{531}/ry^{506} and ry^{606}/ry^{506}, a noncrossover recombinant in which the ry^{531} mutation was converted to wild type (GC), and a noncrossover recombinant with PMS at the site of the ry^{531} mutation (PMS). Two PCRs were performed on each sample, one using an allele-specific primer for the ry^{531} mutation (531) and another using an allele-specific primer for the wild-type sequence at that site (+). (B) Chromatograms from sequencing of PCR products, showing the region from 3731 to 3695 (reverse strand). Arrows below the chromatograms indicate the positions of two polymorphic sites; the site on the left (3723) is G in ry^{531} and T in ry^{606}, and the site on the right (3703) is A in ry^{531} and T in ry^{606}. For the chromatogram at the top, the template was a PCR product from a ry^{531} fly. For the other three, the templates were PCR products from the same CO from an *Msh6* mutant (boxed region of bottom CO in Figure 2, class IV). This CO had PMS at the ry^{531} mutation site. PCR was done using a nonspecific primer (ns), a primer that amplifies the strand with the ry^{531} mutation (531), or a primer that amplifies the strand with the wild-type sequence at that site (+). The chromatograms show PMS at the 3703 site and demonstrate that the base derived from the ry^{531} chromosome (A) is on the strand carrying the ry^{531} mutation. The polymorphism at 3723 has the base from the ry^{531} chromosome (T) on both strands.

both recombinants that transmitted only *ry*– through the germline. This demonstrates that allele-specific PCR is sensitive enough to detect somatic mosaicism even when the germline, which constitutes a significant fraction of the tissues in an adult fly, is entirely *ry*–. This finding, coupled with results from control amplifications in which we detect either allele when it is present in only 20% of the DNA molecules in a sample (the lowest concentration attempted, Figure 1A), suggests that allele-specific PCR detects most or all PMS.

The third assay we used to detect PMS was to sequence non-allele-specific PCR products and examine chromatograms for the presence of double peaks at polymorphic sites (Figure 1B). With this assay, we detected PMS in each of the 19 cases detected by allele-specific PCR of the *ry* mutation sites, as well as in 18 additional recombinants that exhibited PMS at sites of silent polymorphism but not at the mutant sites. There were no cases in which results from one assay were inconsistent with results from another assay.

We did not detect PMS in any of the 165 recombinants (112 COs and 53 NCOs) from wild-type females (Table 1) (BLANTON *et al.* 2005; RADFORD *et al.* 2007), confirming results of earlier experiments that suggested that PMS is exceedingly rare in Drosophila, at least among NCOs from wild-type females (CHOVNICK *et al.* 1971). In contrast, we detected PMS in 37 of 106 recombinants from *Msh6* mutant females, a significantly higher frequency ($P < 0.0001$). The frequency of PMS was significantly lower ($P = 0.0003$) among COs (14 of 66, 21%) than among NCOs (23 of 40, 58%). This result was unexpected, because it suggests a difference in either MMR or hDNA formation between COs and NCOs. Nonetheless, the finding of PMS in both CO and NCO products from *Msh6* mutants confirms that hDNA is indeed an important feature of meiotic recombination in Drosophila, and that MSH6 is involved in the repair of mismatches within this hDNA.

GC and hDNA tracts from *Msh6* mutants are discontinuous: The structure and arrangement of hDNA in recombination products can be used to infer the nature of recombination intermediates. Having successfully created a Drosophila MMR mutant in which we can recover unrepaired hDNA, we sought to examine the recombination events from this mutant to gain insight into the recombination process. We examined the extent of unrepaired hDNA and GC tracts in the COs and NCOs from *Msh6* mutants through PCR and sequencing (Figure 1, MATERIALS AND METHODS). Sequence analysis was considered complete when at least one unconverted polymorphism was found on both ends of the GC or hDNA tracts. In some cases, the end point identified by this method may actually be a patch of restoration repair. This would lead to an underestimation of the extent of GC and hDNA tracts and an underestimation of the level of discontinuity associated with COs and NCOs, but these limitations do not substantially affect the conclusions drawn from this analysis.

Among the 66 COs from *Msh6* mutants, we observed four structural classes (Figure 2): (I) "normal," with a single exchange point (51 COs, 77%); (II) an hDNA

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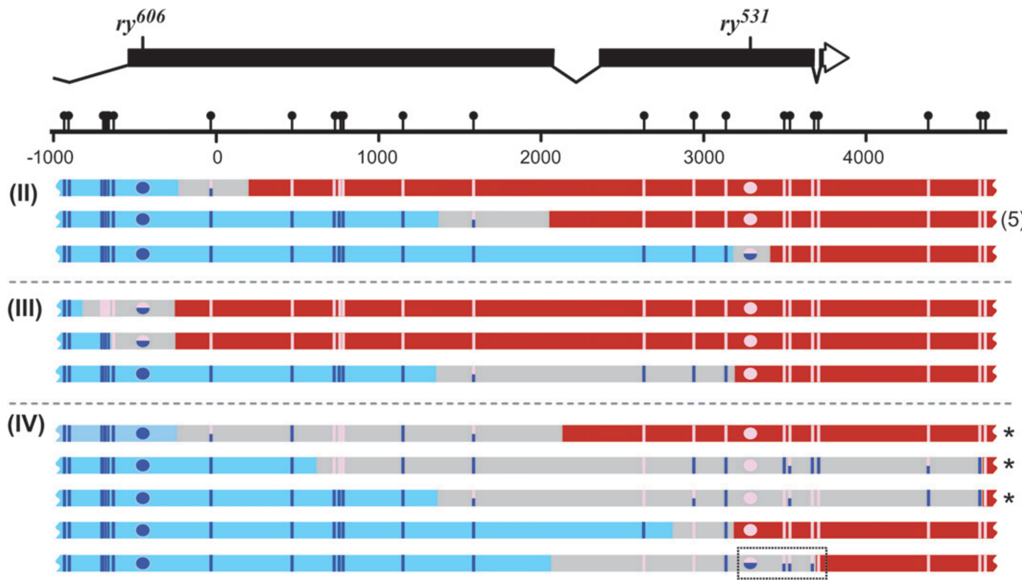

FIGURE 2.—Complex CO events from meiotic recombination in *Msh6* mutants. The schematic at the top shows the *rosy* locus, except for the first exon. Solid regions are coding sequences. The positions of the selected sites corresponding to the ry^{606} and ry^{531} mutations are indicated. Additional polymorphisms are shown as lollipops on the scale bar. These are all single-nucleotide polymorphisms, except for −1029 (not shown here, but see Figure 3) and −685, which are insertions of 1 and 4 bp, respectively, in ry^{531} relative to ry^{606} (see supplemental Table S1 at http://www.genetics.org/supplemental/ for a list of polymorphic sites). The scale is in base pairs, using the coordinate system of BENDER *et al.* (1983). The structures of the complex COs from *Msh6* mutants are diagrammed below. Each bar represents an independent event, with the circles denoting the selected markers (ry^{606} or ry^{531} mutant sites). Gray bars show the region surrounding the recombination event. Blue bars represent sequences derived from the ry^{531} chromosome, and red bars represent sequences from the ry^{606} chromosome. Polymorphisms are marked with blue or pink lines to indicate alleles from the ry^{531} or the ry^{606} parental chromosome, respectively. Polymorphisms that exhibited PMS are marked with half-pink, half-blue lines. Events are grouped according to the numbered classes described in the text. One structure was observed five times, as indicated by the (5) to the right of the structure. Gray asterisks mark events with multiple PMS patches; all were in the *cis* orientation. The chromatograms in Figure 1 are from PCR products corresponding to the boxed region on the bottommost CO. Class I COs are not pictured, but using this scheme they would be depicted as a simple transition from blue to red at a single point. The sites of these transitions are listed in Table 2.

tract at a single point of exchange (7 COs, 10.5%); (III) an hDNA tract separated from a single point of exchange (3 COs, 5%); and (IV) multiple points of exchange, with or without hDNA (5 COs, 7.5%). Class I COs are indistinguishable from the COs we recovered from wild type in that they lacked discontinuities. In addition, the distribution of exchange points across the 3.8-kb region between the *ry* mutations was not significantly different between COs from wild-type females and class I COs from *Msh6* mutants (Table 2). It is evident from this result that most COs do not require MSH6 for hDNA repair; however, it is not clear whether this reflects the existence of an MSH6-independent MMR pathway or whether hDNA is not formed, or is not extensive enough to be detected, in these COs. The remaining three types of COs from *Msh6* are distinct from COs from wild type both in the appearance of unrepaired hDNA (classes II, III, and IV) and in multiple exchange points or discontinuities (class IV).

We also observed four structural classes among 40 NCOs from *Msh6* mutants (Figure 3): (I) normal, with a single, continuous GC tract (9 NCOs, 20%); (II) a single, continuous hDNA tract (4 NCOs, 10%); (III) a single GC tract interrupted by an hDNA tract (10 NCOs, 25%); and (IV) a discontinuous GC tract, with or without hDNA (17 NCOs, 45%). Class I NCOs are similar to NCOs observed from wild type (BLANTON *et al.* 2005; RADFORD *et al.* 2007) in that the GC tracts are continuous. Furthermore, all GC tract lengths observed from Class I NCOs from *Msh6* mutants have also been

TABLE 2

Crossover distribution in wild-type and *Msh6* mutants

Interval	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Size (bp)	424	511	269	31	10	376	443	1057	304	191	164
Wild-type COs	10	12	7	0	0	8	13	32	8	7	1
Msh6 COs	9	3	8	0	1	3	3	16	4	2	2

The 3.8-kb region between the ry^{606} and ry^{531} mutant sites was divided into 11 intervals defined by the 10 polymorphisms in this region (Figure 1, supplemental Table S1 at http://www.genetics.org/supplemental/). Intervals are listed in chromosomal order from left (ry^{606} mutation) to right (ry^{531} mutation). The exchange positions of 105 COs from wild-type females and the 51 class I COs from *Msh6* mutants were determined by PCR and sequencing. The number of COs with an exchange in each interval is given. The distributions are not significantly different from one another ($P = 0.37$).

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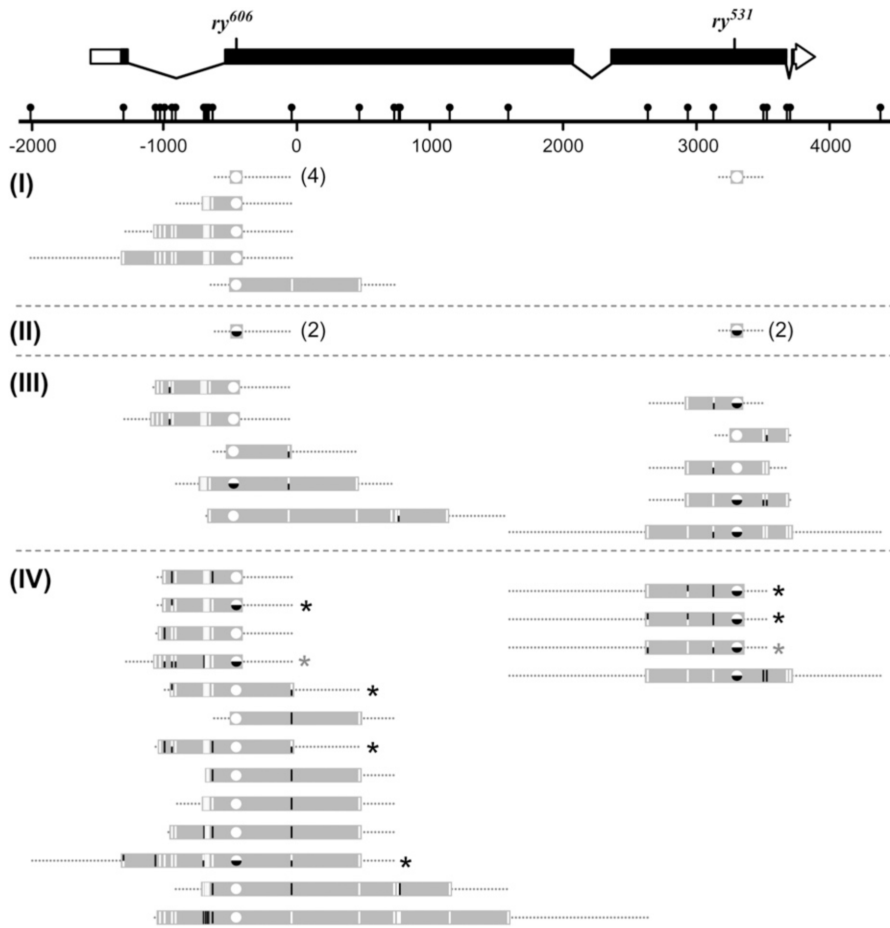

Figure 3.—NCO events from meiotic recombination in *Msh6* mutants. A schematic of the *ry* locus is shown as in Figure 2, but covering a larger region. GC and hDNA tracts of NCOs recovered from *Msh6* mutants are shown below. Shaded bars represent the minimum tract length for each event, and dotted lines the maximum possible length based on the location of the adjacent unconverted polymorphism. The selected sites (ry^{606} for tracts on the left, ry^{531} for tracts on the right) are indicated with circles. Co-converted sites are marked by open vertical bars, and sites within a tract that were not converted (*i.e.*, restored) are marked by solid vertical bars. Asterisks mark events with multiple patches of hDNA; those with solid asterisks have *trans* hDNA. Numbers in parentheses indicate multiple independent events with the same structure.

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observed in NCOs from wild type, although the small number of Class I NCOs from *Msh6* mutants does not allow statistical comparison of mean GC tract lengths. The remaining three classes of NCOs from *Msh6* mutants, which represent the majority of events, are distinct from NCOs from wild type due to the appearance of hDNA (classes II, III, and IV) and the discontinuity of tracts (classes III and IV).

Our results reveal that many recombination events from *Msh6* mutants exhibit discontinuities or "patchiness." We have identified a single discontinuous GC tract from wild type (Blanton *et al.* 2005) and two discontinuous tracts from *mei-9* mutants (Radford *et al.* 2007). In each of these, a long GC tract contains a single site that appeared to undergo restoration repair. We observed many more "patchy" events from *Msh6* mutants, and these were more complex, sometimes including unrepaired sites or multiple discontinuities (Figures 2 and 3), suggesting that this phenomenon is a consequence of *Msh6* mutation.

DISCUSSION

Our current understanding of the molecular mechanism of meiotic recombination is derived mostly from experimental evidence that has been collected in fungi. Although fungi have many advantages that make them amenable to studies of meiotic recombination, and these studies have greatly increased our knowledge of this process, it is important to investigate this process in other model organisms to determine which details are specific to a certain organism and which are universal. For example, Spo11 generates double-strand breaks that initiate meiotic recombination in *S. cerevisiae* (Keeney *et al.* 1997), and orthologs of this protein are essential for meiotic recombination in *Schizosaccharomyces pombe*, *Arabidopsis thaliana*, *C. elegans*, *D. melanogaster*, and *Mus musculus*, indicating that the mechanism of recombination initiation is widely conserved (reviewed in Keeney and Neale 2006). In contrast, the Mus81 endonuclease is required to generate most meiotic crossovers in *S. pombe* and a subset of crossovers in *S. cerevisiae*, but does not appear to be required to generate crossovers in *C. elegans*, Drosophila, or mice (reviewed in Whitby 2005; K. Trowbridge, K. S. McKim, S. Brill and J. Sekelsky, unpublished results), revealing unexpected diversity in the late stages of crossover formation.

One important feature of molecular models of meiotic recombination is the presence of hDNA. Investigation of hDNA in fungi has proven fruitful in many instances; however, these experiments have been limited in the structural detail they provide due to limitations in

the number of heterologies that can be used simultaneously (see Introduction). We have developed a system originally designed by Chovnick and colleagues (Chovnick *et al.* 1970, 1971) that allows us to analyze the molecular structures of recombination events with high resolution in the model organism *D. melanogaster* (Blanton *et al.* 2005; Radford *et al.* 2007). Unrepaired hDNA is almost never detected in wild-type Drosophila, however, so we set out to increase our ability to recover hDNA using our recombination system.

In *S. cerevisiae*, recovery of unrepaired hDNA as PMS is greatly increased in MMR-defective mutants (reviewed in Borts *et al.* 2000). We found that PMS is dramatically increased in Drosophila *Msh6* mutants, which demonstrates that hDNA and the use of MMR to repair heterologies in hDNA are both conserved features of meiotic recombination in this organism. Molecular analysis of the structures of recombination events in *Msh6* mutants revealed a surprising amount of discontinuity in GC and hDNA tracts. There are several possible sources of these discontinuities. If mutations in *Msh6* abolish all meiotic MMR, then the discontinuities in recombination events from these mutants must reveal the underlying structure of hDNA and GC tracts in recombination intermediates and products. This suggests that recombination normally results in the formation of discontinuous hDNA and GC tracts, which are then masked by the action of MMR. Alternatively, loss of MMR may disrupt the normal recombination process in some manner that leads to generation of discontinuous tracts. In mitotically proliferating Drosophila cells, it is thought that repair of double-strand gaps involves multiple rounds of strand invasion, repair DNA synthesis, and dissociation of the nascent strand from the template (McVey *et al.* 2004a). Discontinuous meiotic hDNA and GC tracts could occur if there are multiple cycles, with the sister chromatid used in some cycles and the homologous chromosome in others. This could explain some of the simpler discontinuous events we observed, but additional processes would be necessary to explain the complex events we recovered that contain patches of conversion, restoration, and hDNA. For this reason, we do not believe that the discontinuities we observed are present in recombination intermediates, but rather are the result of some MSH6-independent hDNA repair.

The only other MutS homolog in the Drosophila genome is SPEL1 (MSH2). It is possible that SPEL1 can function in MMR as a homodimer or as a heterodimer with some MutS homolog that has not been identified in the genome sequencing projects; however, in the discontinuous tracts that we observed, closely spaced heterologies are sometimes treated independently (converted, restored, or left unrepaired; Figures 2 and 3), suggesting that canonical MMR is not responsible.

To account for the discontinuities in GC and hDNA tracts that we recovered, we propose that removal of the canonical MMR pathway reveals the action of an alternative repair pathway in which repair tracts are very short. The existence of a short-patch repair pathway in the absence of canonical MMR has been suggested in both *S. cerevisiae* and *S. pombe*, although the proteins involved may not all be conserved between these organisms (Fleck *et al.* 1999; Coic *et al.* 2000). Discontinuous hDNA and GC tracts were also seen in the products of meiotic recombination from mouse $Mlh1^{-/-}$ mutants (Guillon *et al.* 2005), suggesting that short-patch repair may also occur in vertebrates. Some PMS was detected for each type of base–base mismatch, but we did not detect any PMS at sites that would produce an insertion/deletion loop in hDNA (supplemental Table S3 at http://www.genetics.org/supplemental/). It is possible that unpaired loops are efficiently recognized by a short-patch repair system; however, there were other sites, including some for each possible base–base mismatch, at which we never detected PMS (*e.g.*, −679, −668, −667, −636, 1153, and 3723; Figures 2 and 3), suggesting that sequence context may play a role in recognition of mismatches by the short-patch repair system.

According to the model discussed above, the unrepaired hDNA we recovered represents a subset of the hDNA that was formed during recombination, so examination of the PMS events can provide insight into the meiotic recombination process. Among the 14 PMS COs, we obtained 10 (Figure 2, classes II and III) with structures predicted by the two resolution types described in the double-strand break repair (DSBR) model (Szostak *et al.* 1983) (Figure 4). The remaining four PMS COs are not inconsistent with this model, but they cannot be unambiguously assigned to these classes because of the discontinuities that are present. This analysis supports the DSBR model as applicable to Drosophila meiotic recombination.

In the canonical DSBR model, COs and NCOs are alternate outcomes of resolution of the same recombination intermediate; however, several lines of evidence have led to the suggestion that many NCOs are derived from synthesis-dependent strand annealing (SDSA). Both resolution and SDSA predict that only *cis* hDNA may be formed, hDNA in which markers remain linked in the parental orientation (Figure 4). Evidence from *S. cerevisiae*, however, suggests an additional source of NCOs because some events with two tracts of hDNA in opposite orientations are observed, termed *trans* hDNA (Gilbertson and Stahl 1996). Many of the NCO PMS events we characterized had a single tract of hDNA, but there were eight cases with two or three separate regions of hDNA (Figure 3, class IV, asterisks). In six of these, hDNA tracts were in the *trans* orientation (Figure 3, class IV, solid asterisks), suggesting that, as in *S. cerevisiae*, there is a source of NCOs in addition to SDSA and resolution. We also observed three cases in which COs contained two or three separate regions of hDNA (Figure 2, class IV, asterisks); however, these were never in the *trans* orientation.

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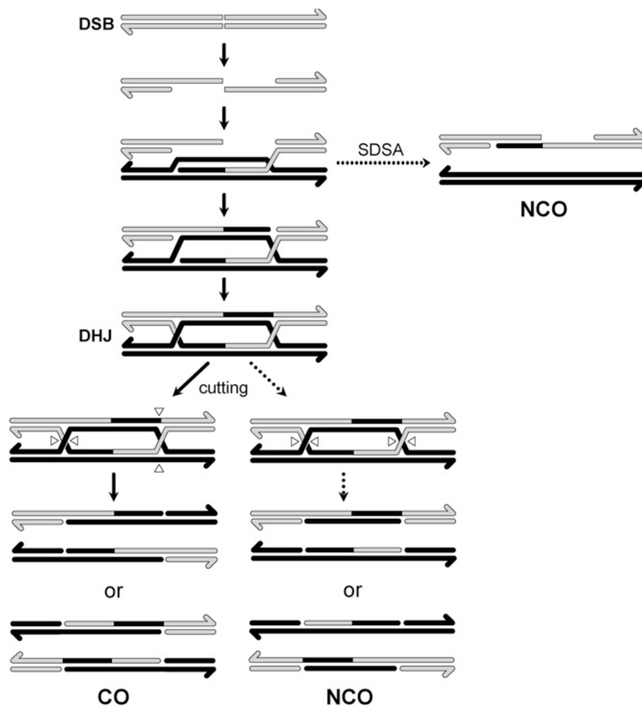

FIGURE 4.—DSBR model for meiotic recombination. According to this model, recombination initiates with the introduction of a double-strand break (DSB) on one chromatid (shaded lines), followed by 5′–3′ resection of the ends to leave 3′ single-stranded overhangs. One 3′ end invades the duplex of a chromatid of the homologous chromosome (solid lines), base pairing with the complementary strand and displacing the other strand as a D-loop. Synthesis follows, primed by the 3′ end of the broken chromosome and using the invaded chromosome as a template. This strand may dissociate and anneal to the second broken end to generate an NCO by SDSA. Alternatively, the strand displaced by synthesis may anneal to the second broken end. Additional synthesis and ligation will then produce a double Holliday junction (DHJ) intermediate. The DHJ is resolved by cutting to generate CO or NCO products. Each Holliday junction may be cut in either of two orientations. Triangles indicate cut sites for one orientation that generates COs and for one that generates NCOs, corresponding to the pairs of products below each. The bottommost pairs of products are generated by cutting each junction in the opposite orientation to that shown by triangles. Proposed NCO pathways are indicated by stippled arrows.

We also noted that COs exhibit PMS less frequently than NCOs in *Msh6* mutants. We have hypothesized that, in *Msh6* mutants, a short-patch repair system can act on mismatches in hDNA, reducing the level of PMS that we observe. While we cannot exclude the possibility that short-patch repair is more active on COs than on NCOs, a more plausible explanation is that less hDNA is present in recombination intermediates that lead to COs. In current models for CO formation, hDNA is formed during strand invasion and during second-end capture (Figure 4). In contrast, many NCOs are proposed to arise from SDSA, in which hDNA is formed during annealing (Figure 4). A possible explanation for our results, therefore, is that strand invasion and second-end capture generate less hDNA than the annealing step of SDSA. This model makes the prediction that, in Drosophila, GC tracts associated with COs are shorter than GC tracts associated with NCOs. Because we recover only one of the four products of meiotic recombination in our experiments, we are unable to analyze GC tracts associated with COs; however, the recovery of both chromatids involved in a recombination event has been accomplished in Drosophila (Chovnick *et al.* 1970), so an investigation of GC tracts associated with COs is possible.

The results reported here underscore the importance of exploiting the unique advantages of different model organisms to understand conserved processes. In particular, studying meiotic recombination in Drosophila provides an opportunity to investigate the length and arrangement of GC and hDNA tracts with high resolution and to use this information to gain insights into functions of various recombination proteins and the molecular mechanism of recombination. To this end, we created a Drosophila MMR mutant that allows recovery of unrepaired hDNA. We demonstrate that discontinuities in GC and hDNA tracts are common in an MMR mutant. The structure of hDNA tracts suggests that some NCOs are derived from a recombination intermediate that contains *trans* hDNA, a result that corroborates existing data in *S. cerevisiae*. Our results also suggest that associated hDNA tracts may differ in length between COs and NCOs. This supports the hypothesis that not all NCOs and COs are alternative outcomes of resolution of a common recombination intermediate.

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LITERATURE CITED

Adams, M. D., M. McVey and J. Sekelsky, 2003 Drosophila BLM in double-strand break repair by synthesis-dependent strand annealing. Science **299:** 265–267.

Allers, T., and M. Lichten, 2001 Intermediates of yeast meiotic recombination contain heteroduplex DNA. Mol. Cell **8:** 225–231.

Bender, W., P. Spierer and D. S. Hogness, 1983 Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the bithorax complex in *Drosophila melanogaster.* J. Mol. Biol. **168:** 17–33.

Blanton, H. L., S. J. Radford, S. McMahan, H. M. Kearney, J. G. Ibrahim *et al.*, 2005 REC, *Drosophila* MCM8, drives formation of meiotic crossovers. PLoS Genet. **1:** 343–353.

Borner, G. V., N. Kleckner and N. Hunter, 2004 Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell **117:** 29–45.

Borts, R. H., and J. E. Haber, 1987 Meiotic recombination in yeast: alteration by multiple heterozygosities. Science **237:** 1459–1465.

Borts, R. H., S. R. Chambers and M. F. Abdullah, 2000 The many faces of mismatch repair in meiosis. Mutat. Res. **451:** 129–150.

Carpenter, A. T. C., 1982 Mismatch repair, gene conversion, and crossing-over in two recombination-defective mutants of *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **79:** 5961–5965.

Chovnick, A., G. H. Ballantyne, D. L. Baillie and D. G. Holm, 1970 Gene conversion in higher organisms: half-tetrad analysis of recombination within the rosy cistron of *Drosophila melanogaster.* Genetics **66:** 315–329.

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Chovnick, A., G. H. Ballantyne and D. G. Holm, 1971 Studies on gene conversion and its relationship to linked exchange in *Drosophila melanogaster.* Genetics **69:** 179–209.

Clark, S. H., A. J. Hilliker and A. Chovnick, 1988 Recombination can initiate and terminate at a large number of sites within the *rosy* locus of *Drosophila melanogaster.* Genetics **118:** 261–266.

Coic, E., L. Gluck and F. Fabre, 2000 Evidence for short-patch mismatch repair in *Saccharomyces cerevisiae.* EMBO J. **19:** 3408–3417.

Denver, D. R., S. Feinberg, S. Estes, W. K. Thomas and M. Lynch, 2005 Mutation rates, spectra and hotspots in mismatch repair-deficient *Caenorhabditis elegans.* Genetics **170:** 107–113.

Fleck, O., E. Lehmann, P. Schar and J. Kohli, 1999 Involvement of nucleotide-excision repair in msh2 pms1-independent mismatch repair. Nat. Genet. **21:** 314–317.

Flores, C., and W. Engels, 1999 Microsatellite instability in Drosophila spellchecker1 (MutS homolog) mutants. Proc. Natl. Acad. Sci. USA **96:** 2964–2969.

Fogel, S., R. K. Mortimer and K. Lusnak, 1981 *The Molecular Biology of the Yeast Saccharomyces*, edited by J. Strathern, E. W. Jones and J. Broach, pp. 289–339. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Foss, H. M., K. J. Hillers and F. W. Stahl, 1999 The conversion gradients at *HIS4* of *Saccharomyces cerevisiae.* II. A role for mismatch repair directed by biased resolution of the recombinational intermediate. Genetics **153:** 573–583.

Gerton, J. L., J. DeRisi, R. Shroff, M. Lichten, P. O. Brown *et al.*, 2000 Global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **97:** 11383–11390.

Gilbertson, L. A., and F. W. Stahl, 1996 A test of the double-strand break repair model for meiotic recombination in *Saccharomyces cerevisiae.* Genetics **144:** 27–41.

Gloor, G. B., C. R. Preston, D. M. Johnson-Schlitz, N. A. Nassif, R. W. Phillis *et al.*, 1993 Type I repressors of *P*-element mobility. Genetics **135:** 81–95.

Guillon, H., F. Baudat, C. Grey, R. M. Liskay and B. de Massy, 2005 Crossover and noncrossover pathways in mouse meiosis. Mol. Cell **20:** 563–573.

Hilliker, A. J., S. H. Clark and A. Chovnick, 1991 The effect of DNA sequence polymorphisms on intragenic recombination in the *rosy* locus of *Drosophila melanogaster.* Genetics **129:** 779–781.

Hoffmann, E. R., and R. H. Borts, 2005 *Trans* events associated with crossovers are revealed in the absence of mismatch repair genes in *Saccharomyces cerevisiae.* Genetics **169:** 1305–1310.

Hoffmann, E. R., E. Eriksson, B. J. Herbert and R. H. Borts, 2005 MLH1 and MSH2 promote the symmetry of double-strand break repair events at the HIS4 hotspot in *Saccharomyces cerevisiae.* Genetics **169:** 1291–1303.

Holliday, R., 1964 A mechanism for gene conversion in fungi. Genet. Res. **78:** 282–304.

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

Jessop, L., T. Allers and M. Lichten, 2005 Infrequent co-conversion of markers flanking a meiotic recombination initiation site in *Saccharomyces cerevisiae.* Genetics **169:** 1353–1367.

Judd, S. R., and T. D. Petes, 1988 Physical lengths of meiotic and mitotic gene conversion tracts in *Saccharomyces cerevisiae.* Genetics **118:** 401–410.

Keeney, S., and M. J. Neale, 2006 Initiation of meiotic recombination by formation of DNA double-strand breaks: mechanism and regulation. Biochem. Soc. Trans. **34:** 523–525.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Kunkel, T. A., and D. A. Erie, 2005 DNA mismatch repair. Annu. Rev. Biochem. **74:** 681–710.

McVey, M., M. D. Adams, E. Staeva-Vieira and J. Sekelsky, 2004a Evidence for multiple cycles of strand invasion during repair of double-strand gaps in Drosophila. Genetics **167:** 699–705.

McVey, M., J. R. LaRocque, M. D. Adams and J. Sekelsky, 2004b Formation of deletions during double-strand break repair in Drosophila DmBlm mutants occurs after strand invasion. Proc. Natl. Acad. Sci. USA **101:** 15694–15699.

Merker, J. D., M. Dominska and T. D. Petes, 2003 Patterns of heteroduplex formation associated with the initiation of meiotic recombination in the yeast *Saccharomyces cerevisiae.* Genetics **165:** 47–63.

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

Modrich, P., and R. Lahue, 1996 Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:** 101–133.

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

Radford, S. J., S. McMahan, H. L. Blanton and J. Sekelsky, 2007 Heteroduplex DNA in meiotic recombination in Drosophila *mei-9* mutants. Genetics **176:** 63–72.

Romans, P., 1980 Effects of purine selection on survival of Drosophila mosaic for xanthine dehydrogenase (XDH) activity. Dros. Inf. Serv. **55:** 132–133.

Sekelsky, J., M. H. Brodsky and K. C. Burtis, 2000 DNA repair in Drosophila. Insights from the Drosophila genome sequence. J. Cell Biol. **150:** F31–F36.

Symington, L. S., and T. D. Petes, 1988 Expansions and contractions of the genetic map relative to the physical map of yeast chromosome III. Mol. Cell. Biol. **8:** 595–604.

Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein and F. W. Stahl, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

Wang, T. F., N. Kleckner and N. Hunter, 1999 Functional specificity of MutL homologs in yeast: evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction. Proc. Natl. Acad. Sci. USA **96:** 13914–13919.

Whitby, M. C., 2005 Making crossovers during meiosis. Biochem. Soc. Trans. **33:** 1451–1455.

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