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# Heteroduplex DNA in Meiotic Recombination in Drosophila *mei-9* Mutants

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ABSTRACT

Meiotic recombination gives rise to crossovers, which are required in most organisms for the faithful segregation of homologous chromosomes during meiotic cell division. Characterization of crossover-defective mutants has contributed much to our understanding of the molecular mechanism of crossover formation. We report here a molecular analysis of recombination in a *Drosophila melanogaster* crossover-defective mutant, *mei-9*. In the absence of *mei-9* activity, postmeiotic segregation associated with noncrossovers occurs at the expense of crossover products, suggesting that the underlying meiotic function for MEI-9 is in crossover formation rather than mismatch repair. In support of this, analysis of the arrangement of heteroduplex DNA in the postmeiotic segregation products reveals different patterns from those observed in Drosophila *Msh6* mutants, which are mismatch-repair defective. This analysis also provides evidence that the double-strand break repair model applies to meiotic recombination in Drosophila. Our results support a model in which MEI-9 nicks Holliday junctions to generate crossovers during meiotic recombination, and, in the absence of MEI-9 activity, the double Holliday junction intermediate instead undergoes dissolution to generate noncrossover products in which heteroduplex is unrepaired.

ACCURATE chromosome segregation during meiosis requires crossovers (COs) between homologous chromosomes, which are generated through meiotic recombination. A number of CO-defective mutants have been identified in model organisms (reviewed in Villeneuve and Hillers 2001; McKim *et al.* 2002). Much of our understanding of the molecular mechanism of meiotic recombination comes from genetic studies of the meiotic phenotypes of these mutants, molecular cloning and identification of the genes affected, and biochemical studies of the properties of the protein products of these genes. These analyses have led to the establishment of the double-strand break repair (DSBR) model for meiotic recombination (Figure 1) (Szostak *et al.* 1983).

COs are an important product of meiotic recombination because they direct the segregation of homologous chromosomes from one another; however, meiotic recombination also gives rise to noncrossover (NCO) products. COs are easily recognized by the exchange of flanking markers, but NCOs can be distinguished from nonrecombinant chromosomes only when accompanied by gene conversion (GC). According to the DSBR model, GC results from the repair of mismatches in heteroduplex DNA (hDNA), DNA in which each strand of the duplex is derived from a different parental chromosome.

In the canonical DSBR model, COs and NCOs are alternate outcomes of resolution of a common recombination intermediate, the double Holliday junction (DHJ) structure (Figure 1). The existence of a class of mutations that reduce the number of COs but not the number of NCOs argues against this feature of the model. This class includes mutations in *MUS81*, *MMS4*, *MSH4*, *MSH5*, and *MLH1* in *Saccharomyces cerevisiae* (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Hunter and Borts 1997; de los Santos *et al.* 2001, 2003) and in *mei-218*, *rec*, and *mei-9* in Drosophila (Carpenter 1982; Blanton *et al.* 2005). Analysis of these mutants suggests that there is a split in the recombination pathway with one branch leading to COs and the other to NCOs; these mutants are defective in the CO-specific branch. In support of this interpretation, most NCOs in *S. cerevisiae* are now thought to be produced by synthesis-dependent strand annealing (SDSA), with the DHJ being resolved primarily into COs (Figure 1) (Allers and Lichten 2001a).

Although the number of NCOs is not decreased in CO-defective mutants, in some cases these mutants produce NCOs with properties that distinguish them from normal NCOs, such as differences in GC tract length or repair of hDNA (Carpenter 1982; Hunter and Borts 1997; Blanton *et al.* 2005). One possible explanation is that these genes encode proteins that function in the CO branch and in the NCO branch, perhaps with different roles in each. It is also possible that these

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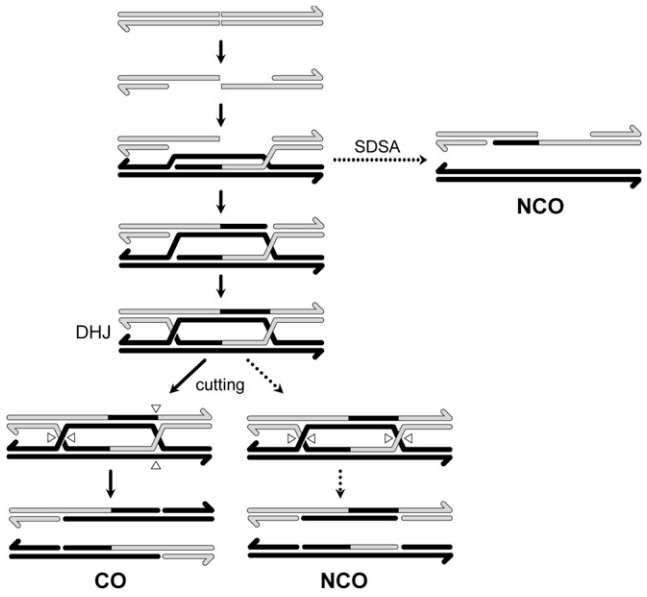


Figure 1.—DSBR model for meiotic recombination According to this model, recombination initiates with the introduction of a double-strand break (DSB) on one chromatid (shaded lines; arrows indicate 3′ ends), followed by 5′–3′ resection of the ends to leave 3′ single-stranded overhangs. One 3′ end invades 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 either dissociates, reannealing to the second broken end to generate an NCO by SDSA, or, alternatively, the D-loop anneals to the second free 3′ end and additional synthesis and ligation produce the double Holliday junction (DHJ) intermediate. The DHJ is resolved by cutting to generate CO or NCO products.

proteins may function solely in the CO branch, and the effect on NCOs is a consequence of an inability to complete the CO pathway (*i.e.*, recombination events that were fated to become COs instead become NCOs). Detailed studies of the properties of NCOs produced by these mutants can provide insights into the molecular mechanism of meiotic recombination as well as specific functions of these proteins.

We recently reported analysis of NCOs in Drosophila *rec* mutants (Blanton *et al.* 2005). The average length of GC tracts among NCOs is lower in *rec* mutants than in wild type, suggesting that REC facilitates repair synthesis during meiotic recombination and that, as is thought to be the case in *S. cerevisiae*, most NCOs in Drosophila arise through SDSA. Mutations in *mei-9* have a different effect on NCOs: they frequently exhibit postmeiotic segregation (PMS) (Romans 1980b; Hilliker and Chovnick 1981; Carpenter 1982, 1984; Bhagat *et al.* 2004). PMS arises from a failure to repair heterologies in hDNA, resulting in sister chromatids containing different sequence information after the first round of postmeiotic replication. With the possible exception of *mei-9*, all mutations that cause PMS in *S. cerevisiae* or Drosophila are in genes encoding proteins known to be involved in mismatch repair (MMR) (reviewed in Borts *et al.* 2000; Radford *et al.* 2007, this issue).

Molecular cloning of *mei-9* revealed that it encodes the Drosophila ortholog of mammalian XPF and *S. cerevisiae* Rad1p (Sekelsky *et al.* 1995), the catalytic subunits of DNA structure-specific endonucleases required for nucleotide excision repair (Bardwell *et al.* 1994; Park *et al.* 1995). This led to the hypothesis that the function of MEI-9 in generating COs is to nick Holliday junctions in DHJ intermediates and that in the absence of MEI-9 these DHJs undergo some process that generates NCOs that are refractory to MMR (Sekelsky *et al.* 1995, 1998). This hypothesis predicts that most NCOs from *mei-9* mutants will be identical to NCOs from wild-type flies, but the subset of NCOs that arise through MEI-9-independent processing of DHJs will exhibit PMS. An alternative hypothesis is that MEI-9 functions both in generating COs and in meiotic MMR and that PMS in *mei-9* mutants is a consequence of defects in MMR. In support of this hypothesis, extracts from embryos mutant for *mei-9* have defects in nick-dependent MMR (Bhui-Kaur *et al.* 1998); however, it is not known how this function relates to MMR during meiosis. If MEI-9 is essential for meiotic MMR, then most or all recombinants from *mei-9* mutants should have PMS.

To distinguish between these two hypotheses, we conducted a molecular analysis of recombination products from *mei-9* mutants. We report here that most NCOs from *mei-9* mutants are indistinguishable from NCOs from wild type in that PMS is absent and GC tracts are continuous and similar in length. The subset of NCOs that did exhibit PMS often had two regions of PMS in the *trans* orientation. Our findings, coupled with findings from previous studies of recombination in *mei-9* mutants and in an MMR-defective mutant, indicate that MEI-9 is not essential for meiotic MMR, although it may function in some specialized repair pathways, and suggest that NCOs with PMS arise from recombination events that were unable to become COs in the absence of MEI-9 activity.

## MATERIALS AND METHODS

For *mei-9* mutants, 120 virgin females of genotype *y mei-9$^a$ f; kar ry$^{606}$/ry$^{531}$ cv-c* were crossed to 30 males of genotype *y/Dp(1;Y)y$^+$; kar ry$^{506}$ cv-c*. For wild type, 20–30 females and 10 males were used. Crosses were set up in bottles containing 25 ml of standard food medium and placed at 25°. After 3 days, flies were transferred to fresh media to establish a second brood, and purine was added to the first brood bottles in the amount of 0.75 ml of 0.15, 0.18, or 0.20% (w/v) in water. This amount corresponds to 1.1 mg (9.4 μmol), 1.35 mg (11.2 μmol), and 1.5 mg (12.5 μmol) of purine per bottle. One of every 25 bottles was left untreated and adult progeny were counted to estimate the number of larvae screened. Purine dosage did not grossly affect the recovery of PMS events: we recovered 1 PMS event of 275,000 screened at 0.15%, 2 of 525,000 at 0.18%, and 2 of 550,000 at 0.20%. In contrast, the

number of *ry* mutants that escaped killing by purine was strongly affected by purine dosage: escapers increased ~10-fold between 0.18 and 0.15% (data not shown).

To determine whether a recombinant chromosome is CO or NCO, visible markers flanking *ry* (*kar*, which is 0.3 map units proximal to *ry* on the $ry^{606}$ chromosome, and *cv-c*, which is 2.1 map units distal to *ry* on the $ry^{531}$ chromosome) were scored in progeny surviving purine selection. Recombinant progeny were mated to *kar* $ry^{506}$ *cv-c* flies of the opposite sex to detect mosaicism via germline transmission. After mating, each recombinant fly was homogenized in buffer containing proteinase K, as described (Gloor *et al.* 1993), and hDNA and GC tracts were determined by PCR amplification and sequencing.

To detect PMS, allele-specific PCR primers for several polymorphisms were used, as in Radford *et al.* (2007). Amplification with both allele-specific primers indicates PMS at that polymorphism. Each set of allele-specific PCR reactions included positive and negative controls, as well as 1:4 mixtures of DNA from both alleles to simulate a mosaic in which one allele is present in only 20% of the DNA molecules. Allele-specific PCR products were purified and sequenced to determine the length and arrangement of hDNA tracts. Additional non-allele-specific primers were also used to detect PMS. PCR products were sequenced in bulk and the chromatogram was examined for double peaks. Sequences of allele-specific primers, PCR conditions, and an example of mapping of an hDNA tract are given in Radford *et al.* (2007).

For one event, it was not possible to design allele-specific primers to confirm the PMS and map the hDNA tracts because of low sequence complexity in the region. Instead, non-allele-specific primers were used to amplify the region, and the product was cloned into a convenient vector for amplification in *Escherichia coli*. At least one clone representing each strand of the hDNA region was sequenced.

Mean GC tract lengths and statistical comparisons were calculated as described in Blanton *et al.* (2005). Frequency comparisons were made using Fisher's exact test with two-tailed *P*-values, computed by Instat 3.05 (GraphPad software).

## RESULTS

To distinguish between models for the role(s) of MEI-9 in meiotic recombination, we recovered 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 involved in purine metabolism and is required for normal eye pigmentation. Females *trans*-heterozygous for $ry^{606}$ and $ry^{531}$, point mutations separated by 3.8 kb, were crossed to males homozygous for $ry^{506}$, which deletes much of the gene. Rare rosy+ recombinants were selected by adding purine to the food during larval development. Among recombinants, COs and NCOs are distinguished from one another using markers flanking *ry* (see materials and methods for details). In female meiosis, only one of the two chromatids involved in any recombination event enters the oocyte, so it is not possible to determine whether a CO has an associated GC tract. In contrast, NCOs are recovered only when accompanied by a GC tract that spans one *ry* mutation. Hence, the COs described here may or may not be associated with GC (or PMS), but the NCOs must be associated with GC (or PMS).

**Most NCOs from *mei-9* mutants do not exhibit PMS:** PMS results when mismatches in hDNA are not repaired during meiosis. When unrepaired hDNA is present in a gamete, DNA replication in the first zygotic S phase produces sister chromatids that differ in sequence. Segregation of these sisters at the first mitosis results in daughter cells that have different sequences where the hDNA was. In a metazoan such as Drosophila, PMS manifests as a mosaic individual, in which some cells have the sequence from one strand of the recombinant chromatid and other cells have the sequence from the other strand. Most of the polymorphisms used in this study do not cause a visible mutant phenotype, so these mosaics can be detected only by molecular methods. Although the two *ry* point mutations do cause a visible mutant phenotype (rosy-colored eyes), XDH is secreted and diffuses throughout the developing larva, allowing *ry*+//*ry*− mosaics that survive purine treatment to develop into adults that are rosy+ in eye color (Romans 1980a); therefore, mosaicism for the mutant sites also cannot be detected visibly in the recombinant fly. We used three assays to screen rosy+ adults for mosaicism: germline sampling, allele-specific PCR, and examination of chromatograms for double peaks after sequencing non-allele-specific PCR products in bulk (Radford *et al.* 2007). Germline sampling can detect PMS only at the *ry* mutant sites and does not detect all *ry*+//*ry*− mosaics (Carpenter 1982; Radford *et al.* 2007), but PCR-based assays provide a more sensitive method to detect PMS at the mutant sites and also at silent polymorphisms.

We previously reported analysis of 81 COs and 31 NCOs from wild-type females (Blanton *et al.* 2005). We have now recovered an additional 31 COs and 22 NCOs (Table 1). We screened 1.4 million larvae from *mei-9* mutant females and recovered 5 COs and 32 NCOs. This represents a 90% decrease in COs and a 60% increase in NCOs compared to wild type, similar to previously published findings (Romans 1980b; Carpenter 1982).

Using genetic approaches, Chovnick *et al.* (1971) found PMS to be exceedingly rare among NCOs from wild-type females. Our molecular analyses gave similar results: we did not detect PMS in any of the 112 COs or 53 NCOs from wild-type females (Table 1) (Blanton *et al.* 2005). We did not detect PMS in any of the five COs from *mei-9* mutant females; however, 5 of 32 NCOs exhibited PMS. This frequency of PMS in NCOs from *mei-9* mutants (16%) is significantly higher than that in NCOs from wild type ($P = 0.0061$). We previously found that meiotic recombination in an MMR mutant results in frequent PMS: we detected PMS in 14 of 66 COs (21%) and 23 of 40 NCOs (58%) derived from females mutant for *Msh6* (Radford *et al.* 2007). The rate of PMS among NCOs from *mei-9* mutants is significantly lower than that in NCOs from *Msh6* mutants ($P = 0.0005$). These results do not support the hypothesis that MEI-9 is essential for meiotic MMR.

**TABLE 1**

**Intragenic recombination in wild-type, *Msh6*, and *mei-9* mutants**

| Genotype | Progeny screened | Crossovers | | | Noncrossovers | | |
|---|---|---|---|---|---|---|---|
| | | *n* | Frequency | PMS (%) | *n* | Frequency | PMS (%) |
| Wild type[a] | 3,710,000 | 112 | $3.0 \times 10^{-5}$ | 0 | 53 | $1.4 \times 10^{-5}$ | 0 |
| *Msh6*[b] | 1,775,000 | 67 | $3.8 \times 10^{-5}$ | 21 | 42 | $2.4 \times 10^{-5}$ | 58 |
| *mei-9* | 1,405,000 | 5 | $0.36 \times 10^{-5}$ | 0 | 32 | $2.3 \times 10^{-5}$ | 16 |

[a] Includes data from Blanton *et al.* (2005).
[b] Data are from Radford *et al.* (2007).

**Most non-PMS NCOs from *mei-9* mutants are indistinguishable from NCOs from wild type:** The relatively low frequency of PMS among NCOs from *mei-9* mutants indicates that MEI-9 is not absolutely required for meiotic MMR. The five NCOs with PMS may represent recombination events that were fated to become COs, but in the absence of MEI-9 became NCOs through a pathway that does not involve MMR. This hypothesis predicts that the NCOs without PMS will be identical to NCOs from wild-type females. An alternative hypothesis is that MEI-9 has a function in generating crossovers and a separate, limited function in meiotic MMR. This hypothesis predicts that some recombinants from *mei-9* mutants will be similar to those from an MMR mutant. To test these hypotheses, we used the multiple polymorphisms between the two *ry* alleles to map GC and hDNA tracts.

We previously described GC tracts from 29 NCOs from wild-type flies and found that, with one exception, all tracts were continuous (Blanton *et al.* 2005). We mapped an additional 22 GC tracts from wild-type females and found that all were continuous (Figure 2). We also mapped the 27 non-PMS GC tracts from *mei-9* mutants. Two discontinuities were found, both at the −937 polymorphism (Figure 2, asterisks). The frequency of discontinuity is not significantly different

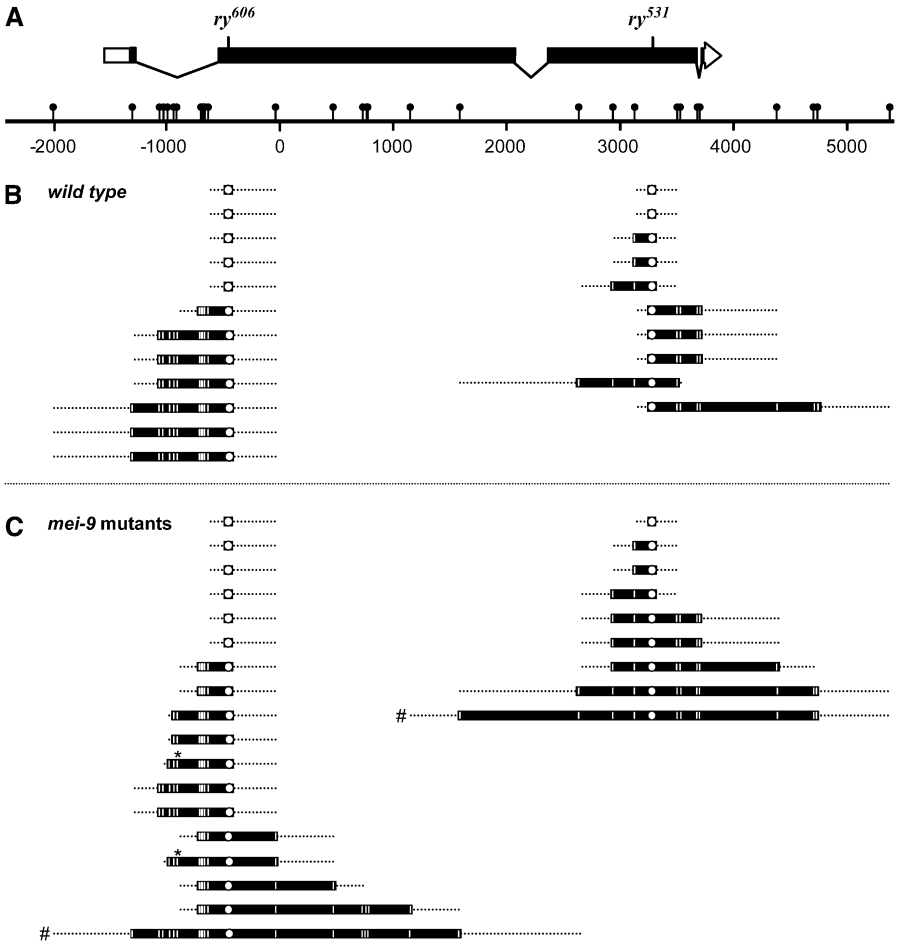


Figure 2.—GC tracts from wild-type and *mei-9* mutants. (A) Schematic of the *rosy* locus. Intron/exon structure is shown, with coding sequences as solid areas. The positions of the selected sites corresponding to the $ry^{606}$ and $ry^{531}$ chromosomes are indicated. Additional polymorphisms are indicated as lollipops on the scale bar. These are all single-nucleotide polymorphisms, except for −1029 and −685, which are insertions of 1 and 4 bp, respectively, in $ry^{531}$ relative to $ry^{606}$. The scale is in base pairs, using the coordinate system of Bender *et al.* (1983). (B and C) Tract lengths observed in NCOs recovered from wild-type (B) and *mei-9* mutants (C). Each bar represents an independent event, with the circle denoting the selected marker ($ry^{606}$ or $ry^{531}$ mutant sites). Solid bars represent the minimum tract length for each event, with coconverted sites marked by the vertical lines within the bars. Dotted lines represent the maximum tract length possible based on the next unconverted polymorphism. The asterisks mark two instances in which conversion at the −937 polymorphism was discontinuous (see materials and methods). Pound signs mark the two unusually long GC tracts (see results).

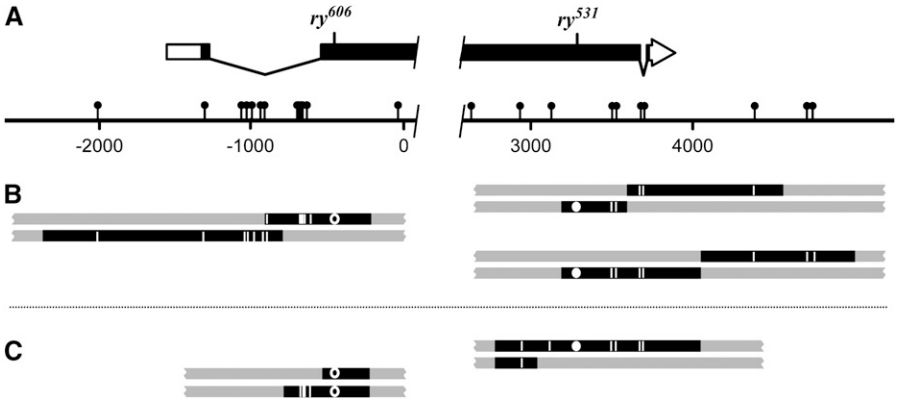


FIGURE 3.—hDNA tracts from meiotic recombination in *mei-9* mutants. (A) Schematic of the *rosy* locus. The symbols are the same as in Figure 2, but only the left and right ends of the gene are shown. (B) Three events with *trans* hDNA. Each pair of bars represents the two strands of a recombinant chromosome with unrepaired hDNA. Solid segments are sequences derived from the homologous chromosome, with vertical hatches showing positions of polymorphisms within hDNA and GC tracts. The position of the $ry^{606}$ point mutation is denoted by an open circle and that of $ry^{531}$ by a solid circle. The ends of the solid segments are drawn at the midpoints between the last included polymorphism and next excluded polymorphism. In all three cases, hDNA on each side was extensive (245–1530 bp) and included at least three polymorphisms. The event that includes $ry^{606}$ showed full conversion of a single polymorphism between regions of hDNA. (C) Two events with a single hDNA tract. In both cases, a single, fully converted polymorphism is present at one end of the tract.

between wild type and *mei-9* mutants ($P = 0.6$). In contrast, 70% of NCOs from *Msh6* mutants show evidence of discontinuities (RADFORD *et al.* 2007), which is significantly different from both wild type and *mei-9* ($P < 0.0001$). Thus, with respect to continuity, GC tracts from *mei-9* mutants are indistinguishable from those from wild-type females, but are unlike those from an MMR mutant.

We also determined mean GC tract length among non-PMS NCOs. Recombination at *ry* is thought to initiate throughout the gene, rather than at one end (CLARK *et al.* 1988); therefore, our experimental design selects for longer GC tracts, since longer tracts are more likely to cross a *ry* mutant site. To estimate the mean tract length in the absence of such selection (unselected tract length), we employed a statistical analysis developed previously (HILLIKER *et al.* 1994; BLANTON *et al.* 2005). The unselected mean GC tract length in NCOs is 425 bp for wild-type females and 509 bp for *mei-9* females. The difference is statistically significant ($P = 0.03$). Closer inspection of the GC tracts from *mei-9* mutants reveals two tracts that are much longer than the others (Figure 2, pound signs). Analysis of actual (selected) tract lengths, taking the end of each tract to be the midpoint between the last polymorphism contained within the tract and the nearest polymorphism excluded from the tract, shows that these two long tracts are more than two standard deviations greater than the mean (mean = 1076 bp, standard deviation = 1026 bp, lengths > 3700 bp). Excluding these two aberrant tracts, the mean unselected length of GCs from *mei-9* mutants is 417 bp, which is not significantly different from the wild-type length ($P = 0.8$). This suggests that most non-PMS NCOs from *mei-9* mutants are indistinguishable from NCOs from wild type in terms of GC tract length. These results also raise the possibility that loss of MEI-9 activity may lead to very long GC tracts in some instances.

Taken together, our results demonstrate that most NCOs from *mei-9* mutants (25 of 32; 78%) are indistinguishable from NCOs from wild type. This finding suggests that most or all NCOs in wild-type flies arise through a MEI-9-independent pathway, providing further support for a model for meiotic recombination in which COs and NCOs arise from separate branches of the meiotic recombination pathway.

**PMS NCOs from *mei-9* mutants exhibit *trans* hDNA:** We sequenced PCR products from the mosaic flies and their progeny (see MATERIALS AND METHODS) to determine the arrangement of hDNA in the PMS NCOs from *mei-9* mutants. We classify the five PMS events from *mei-9* mutants into two types (Figure 3). In the first type (three events, Figure 3B), there are two adjacent hDNA tracts in the *trans* configuration. In one of these, a single site between the two hDNA tracts is fully converted. In the second type (two events, Figure 3C), there is a single hDNA tract adjacent to a single site that is fully converted.

A prediction of models in which MEI-9 participates in meiotic MMR is that the PMS NCOs from *mei-9* mutants will resemble those from *Msh6* mutants. A striking feature of PMS NCOs from *Msh6* mutants is that tracts were frequently discontinuous. We classify an event as discontinuous if there are three or more regions of GC, restoration, and hDNA. Of 18 PMS NCOs from *Msh6* mutants that spanned three or more polymorphisms, 17 (94%) were discontinuous (RADFORD *et al.* 2007). In contrast, of the 5 PMS NCOs from *mei-9* mutants (all of which spanned at least six polymorphisms), only one was discontinuous (Figure 3B, event on the left). Thus, PMS NCOs from *mei-9* mutants are structurally dissimilar from PMS NCOs from an MMR mutant, strengthening our conclusion that MEI-9 is not an essential component of the canonical MMR pathway that operates during meiotic recombination.

## DISCUSSION

In this study, we set out to conduct a careful analysis of meiotic gene conversion and postmeiotic segregation

in *mei-9* mutants. Insights into meiotic functions of MEI-9 are obtained from consideration of the frequency of PMS, the structure of hDNA in recombination products that exhibited PMS, and comparisons with PMS events from a mutant defective in MMR.

**The frequency of PMS in *mei-9* mutants:** We found PMS in only 5 of 32 (16%) NCOs from *mei-9* mutants (Table 1). This is a lower frequency than that in previous studies, in which the frequency of PMS in NCOs from *mei-9* mutants was 60–100% (Romans 1980b; Carpenter 1982; Bhagat *et al.* 2004). We considered several possible explanations for the different results. First, we would observe a lower frequency of PMS if mosaic flies did not survive purine selection as efficiently in our experiments as in previous studies. The degree to which *ry*+//*ry*− mosaic larvae survive purine selection is dependent on the dose of purine used (Romans 1980a). It is not possible to compare purine doses used in experiments in different laboratories, because the final concentration of purine depends on the volume of food in each bottle, which is usually not specified. We used three different amounts of purine: 1.1 mg, 1.35 mg, and 1.5 mg per bottle (see materials and methods). There was no difference in frequency of PMS events within this range. There was, however, a 10-fold increase in survival of *ry*− larvae between the two lowest doses, suggesting that the purine selection we employed was not a substantial barrier to recovery of *ry*+//*ry*− mosaics.

A second possible source of the difference in PMS frequency in our experiments *vs.* previous studies is that the methods we employed (germline sampling and molecular assays) gave false negatives or the methods used previously (germline sampling and staining of adult tissues for XDH activity) gave false positives. In previous studies (Romans 1980b; Carpenter 1982; Bhagat *et al.* 2004), PMS was not detected in recombination events from wild-type females, suggesting that the high frequencies of PMS reported for recombination events from *mei-9* mutants did not result from false positives. Conversely, several considerations suggest that the low frequency of PMS we found is not due to false negatives. First, controls run alongside every allele-specific PCR reaction (see materials and methods) gave the expected results, and we routinely detected the mutant allele when it comprised only 20% of the available template molecules (the lowest concentration tested). Second, results from each of the three methods we used were internally consistent (*e.g.*, the single rosy+ recombinant that transmitted a *ry*− chromosome through the germline was also classified as PMS in both molecular assays we employed). Third, using identical methodologies, we found high levels of PMS in recombination events from *Msh6* mutants (Radford *et al.* 2007), indicating that these methods are efficient in detecting mosaicism.

On the basis of the arguments presented above, we conclude that the low frequency of PMS in our experiments is not an artifact resulting from inability to recover mosaic flies or inability to detect mosaicism. Rather, our results appear to reflect a real difference in PMS frequency, presumably due to other differences in experimental design. Although the different studies all used the genetically null mutation *mei-9*$^{a}$, which is a point mutation predicted to destroy nuclease activity (Yildiz *et al.* 2004), we used different *ry* alleles than did Romans (1980b) and Carpenter (1982). The alleles they used (*ry*$^{5}$ and *ry*$^{41}$) are deletions of 19 and 3 bp, which generate small insertion–deletion loops in hDNA. In contrast, the alleles we used (*ry*$^{606}$ and *ry*$^{531}$) are missense mutations that generate base–base mismatches in hDNA. In *S. cerevisiae*, loops are repaired by different proteins than base–base mismatches (Marsischky *et al.* 1996). Importantly, the *S. cerevisiae* ortholog of MEI-9 (Rad1) has been directly implicated in the repair of loops in meiotic hDNA, but no role in repair of base–base mismatches has been identified (Kirkpatrick and Petes 1997; Kearney *et al.* 2001; Stone and Petes 2006). If this aspect of meiotic MMR is conserved in Drosophila, then the relatively high rates of PMS reported by Romans and by Carpenter may be due to defects in loop repair. Our alleles did include two insertion–deletion heterologies: *ry*$^{531}$ has an insertion of 1 bp at −1029 and an insertion of 4 bp at −685 relative to *ry*$^{606}$. We did not find an increased frequency of PMS at these sites relative to single-nucleotide polymorphisms; however, nearby base–base mismatches (6 and 14 bp flanking the 4-bp insertion and 33 and 32 bp flanking the 1-bp insertion) are expected to stimulate efficient repair of the loops by canonical long-tract MMR (Detloff and Petes 1992).

The *ry* alleles we used also differed from those used by Romans and by Carpenter in the level of sequence heterology. There are no sequence differences between *ry*$^{5}$ and *ry*$^{41}$ other than the small deletions at the mutant sites (S. J. Radford, unpublished data). In contrast, we chose alleles that differed from one another at many sites (~0.4% heterology, Figure 2A) so that we could map conversion and PMS tracts with high resolution. In *S. cerevisiae*, the presence of even a few mismatches decreases the rate of meiotic recombination dramatically (Borts and Haber 1987), but mutations in MMR genes restore normal rates of recombination (Chambers *et al.* 1996; Hunter *et al.* 1996). Drosophila differs in that high levels of heterology do not affect the frequency of meiotic recombination (Hilliker *et al.* 1991). Likewise, loss of MMR does not increase the frequency of meiotic recombination between highly polymorphic chromosomes, although it does allow recovery of recombination events that would have been lost in our selection scheme due to repair of hDNA restoring a mutant allele (Radford *et al.* 2007). In the experiments of Romans and Carpenter, the only heterology in hDNA crosses would be the small unpaired loop caused by the deletion mutation, whereas recombination between the alleles

we used generates hDNA with multiple base–base mismatches. It is possible that these numerous mismatches provide greater opportunity for recruitment of MMR machinery in *mei-9* mutants, leading to the lower frequency of PMS that we observed.

The relatively low frequency of PMS in our experiments compared to previous experiments may be due to one or more of the reasons discussed above; however, the source of the difference does not affect the data and interpretations described below.

**MEI-9 in repair of meiotic hDNA:** We used identical conditions and methodologies to assess the occurrence and structural properties of PMS events from *mei-9* mutants and *Msh6* mutants (Radford *et al.* 2007). The frequency of PMS among NCOs from *mei-9* mutants was significantly lower than the frequency among NCOs from *Msh6* mutants (Table 1), leading us to conclude that MEI-9 is not essential for meiotic MMR of the base–base mismatches present in these experiments. It remains possible that MEI-9 has one or more specialized roles in repair of meiotic hDNA. As discussed above, MEI-9 may be important in repair of unpaired loops in hDNA. It is also possible that MEI-9 is required for MMR of recombination intermediates and products formed during certain NCO pathways, such as DHJ dissolution (see below), because DNA structures are formed that require the endonuclease function of MEI-9 for MMR access. A third possible role for MEI-9 is in short-patch repair. The high frequency of discontinuity among PMS NCOs from *Msh6* led us to propose the existence of a short-patch repair system that operates when canonical MMR is compromised (Radford *et al.* 2007). Only one PMS NCO from *mei-9* was discontinuous (Figure 3B, event on left). The structure of this chromatid—GC at a single polymorphic site between two tracts of hDNA in the *trans* orientation—suggests that the region of GC is due to "early MMR" (see below), rather than short-patch repair. The apparent absence of short-patch repair in NCOs from *mei-9* mutants may indicate that MEI-9 is required for this process. In *Schizosaccharomyces pombe*, there is evidence for a short-patch excision repair pathway that operates on recombination intermediates and that requires the ortholog of MEI-9, Rad16 (Fleck *et al.* 1999). The lack of short-patch repair in *mei-9* mutants could also be due to the presence of MSH6, if binding of MSH6 complexes to mismatches prevents access by short-patch repair proteins. This hypothesis does not exclude a role for MEI-9 in short-patch repair.

**The origin of PMS in *mei-9* mutants:** Examination of the structure of hDNA in NCOs associated with PMS from *mei-9* mutants provides insights into possible functions for MEI-9 in meiotic recombination. Three of the five PMS events we recovered from *mei-9* mutants had two tracts of hDNA in the *trans* orientation (Figure 3B), and two had a single tract of hDNA adjacent to a single site that was fully converted (Figure 3C). It is possible that these latter two events had *trans* hDNA that went undetected. Tracts of hDNA that do not include a polymorphism cannot be detected. On the basis of the nearest polymorphism adjacent to the site of full GC, the second region of hDNA would have to have been $<304$ bp in one case and $<424$ bp in the other. These lengths are both within two standard deviations of the mean hDNA tract length computed using the midpoint between polymorphisms contained and excluded within each of the eight hDNA tracts detected (mean = 805 bp, standard deviation = 381 bp); therefore, we cannot exclude the possibility that in these two cases there was a second hDNA tract that did not include any heterologies.

We may also fail to detect *trans* hDNA if one tract undergoes repair that restores the sequence originally on that chromatid. If each of the tracts in a molecule with *trans* hDNA can be repaired independently, and there is no bias toward restoration repair, we would predict the occurrence of events in which one of the hDNA tracts underwent conversion repair to generate an extensive region of full GC adjacent to a region of hDNA. We did not recover any events with this structure; however, given the small sample size ($n = 2$), failure to observe such events cannot be interpreted as evidence against the hypothesis that the two tracts might be repaired independently. Independent repair of *trans* hDNA tracts could also result in long GC tracts if both tracts are repaired toward conversion. This is a possible explanation for the two unusually long GC tracts we observed among NCOs from *mei-9* mutants (Figure 2, pound signs).

Three of the five PMS events from *mei-9* mutants (one from the first class and both from the second class) had a tract of full gene conversion (Figure 3). In all three cases, the GC tract included only a single polymorphic site. A region of full conversion would result if initiation of recombination involves formation of a double-strand gap or if there is partial repair of hDNA. In *S. cerevisiae* meiotic recombination, mismatches formed during the initial strand invasion are thought to undergo "early" repair (*i.e.*, repair occurs during strand invasion or synthesis, Figure 1), whereas mismatches further away from the site of initiation are thought to be repaired later in the recombination process (Detloff *et al.* 1992; Alani *et al.* 1994; Foss *et al.* 1999; Hillers and Stahl 1999; Stahl and Hillers 2000). Meiotic recombination in *S. cerevisiae* is initiated at hotspots, which are frequently in promoter regions (Gerton *et al.* 2000), so early repair results in higher rates of GC for markers near the 5′ end of the gene. In Drosophila, recombination at *ry* is thought to initiate and terminate throughout the gene rather than at a hotspot at one end (Clark *et al.* 1988; Radford *et al.* 2007), so we cannot determine whether the sites of full GC are near the site of initiation. Nonetheless, the positions of the sites of full GC—between two tracts of *trans* hDNA in one event and at the end of a single hDNA tract in two events—are consistent

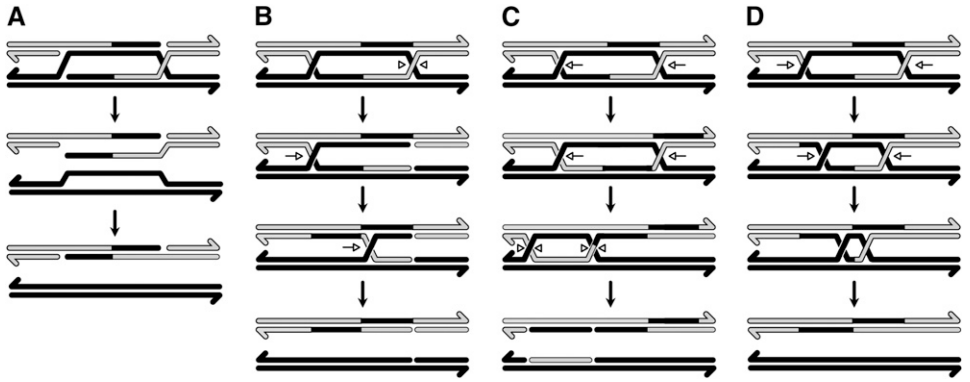


Figure 4.—Models for the formation of *trans* hDNA. (A) Two-ended SDSA. According to the canonical synthesis-dependent strand annealing (SDSA) model (Formosa and Alberts 1986), after strand invasion and synthesis the nascent strand dissociates from the template and anneals to the single-stranded overhang on the other side of the DSB. In two-ended SDSA, both nascent strands dissociate prior to ligation and reanneal to the unresected ends of the break. (B) Single-junction cutting and branch migration. One Holliday junction in the DHJ is cut (arrowheads) and the other undergoes branch migration (arrows) past the nicks. (C) Branch migration and resolution. Both Holliday junctions in the DHJ undergo branch migration in the same direction, past the region of strand invasion and synthesis. Resolution then occurs by cutting. (D) DHJ dissolution. The two Holliday junctions undergo branch migration toward one another, after which the two chromatids are decatenated. All four models predict that one chromatid will contain *trans* hDNA, but only DHJ dissolution results in a product that does not contain nicks.

with the hypothesis that these short conversion tracts arose from early repair.

Formation of *trans* hDNA is not consistent with models in which recombination is initiated by nicks, as in the models proposed by Holliday (1964) and by Meselson and Radding (1975). Rather, *trans* hDNA is consistent with models in which recombination is initiated by a double-strand break (DSB), because this allows both strands of the cut duplex to receive information from the homologous chromosome (Figure 1). In *S. cerevisiae* and *S. pombe*, the DSBR model is supported by physical detection of DSBs and key recombination intermediates (Collins and Newlon 1994; Schwacha and Kleckner 1994, 1995; Cervantes *et al.* 2000; Allers and Lichten 2001b). This type of analysis is not possible in Drosophila, since recombination occurs asynchronously in a small subset of cells in the germline; however, many of the proteins involved in the early stages of meiotic recombination in fungi are conserved in Drosophila, including an ortholog of the protein that catalyzes DSB formation in yeast (McKim and Hayashi-Hagihara 1998). Our finding of *trans* hDNA provides molecular support to strengthen the case made by protein conservation for the application of the DSBR model to Drosophila meiotic recombination.

Following initiation by a DSB, four possible sources of *trans* hDNA can be envisioned (Figure 4, A–D): (A) two-ended SDSA; (B) cutting of the DHJ intermediate at a single HJ followed by branch migration of the second HJ through the nick; (C) branch migration of both HJs past the point of initial resection, followed by resolution by HJ nicking; and (D) branch migration of both HJs toward one another, followed by decatenation by a topoisomerase (DHJ dissolution). If the two events with a single hDNA tract did not actually have *trans* hDNA, they may have arisen through SDSA or through resolution of a DHJ intermediate by nicking (Figure 1).

Our results indicate that MEI-9 is not an essential component of the canonical MMR pathway during meiotic recombination. As described above, we cannot exclude the possibility that the occurrence of PMS in *mei-9* mutants may result from specialized roles for MEI-9 in MMR; however, PMS might also be an indirect consequence of failure to generate COs in the absence of MEI-9, leading to the use of a pathway that generates NCOs that are refractory to MMR. In proliferating cells, MMR is stimulated by nicks (reviewed in Kunkel and Erie 2005). Similarly, it has been suggested that MMR that occurs later in meiotic recombination is directed by nicks introduced during DHJ resolution (Foss *et al.* 1999; Hillers and Stahl 1999; Stahl and Hillers 2000). Among the possible sources of *trans* hDNA enumerated above, DHJ dissolution (Figure 4D) is unique in that nicks are not present after the molecules participating in recombination are resolved into independent duplexes. The absence of nicks might make the products of dissolution resistant to MMR.

Consideration of the structures of hDNA in PMS NCOs provides support for the hypothesis that MEI-9 is required to resolve DHJs into COs and that in the absence of MEI-9 these intermediates undergo dissolution to generate NCOs with *trans* hDNA (Figure 4D); however, we also detected *trans* hDNA in PMS events from *Msh6* mutants (Radford *et al.* 2007). As described above, there are several possible sources of *trans* hDNA. We propose that *trans* hDNA in NCOs from *mei-9* mutants comes from DHJ dissolution. It is possible that dissolution is also the source of *trans* hDNA in NCOs from *Msh6* mutants. For this to be true, DHJ dissolution would have to be a significant source of NCOs even in the presence of MEI-9. If the products of dissolution are always refractory to MMR, then we would expect to recover PMS events with *trans* hDNA from wild-type females. The rarity of PMS in wild-type females stands in opposition to this prediction. Alternatively, the products of dissolution might be subject to MMR, but the MEI-9 endonuclease may be required to make nicks to allow MMR, since these products do not already have nicks

present. Given the similar frequencies of *trans* hDNA in *mei-9* and *Msh6* mutants (3/32 *vs.* 6/40, $P = 0.7$), we cannot exclude this possibility. Another explanation for the presence of *trans* hDNA among NCOs from *Msh6* mutants, however, is that this *trans* hDNA arises from one of the other mechanisms described above (Figure 4, A–C). This hypothesis does not exclude the possibility that PMS NCOs from *mei-9* mutants arise through DHJ dissolution.

In conclusion, our finding that PMS events from *mei-9* mutants often have *trans* hDNA supports the basic features of the DSBR model for meiotic recombination and is consistent with a role for MEI-9 in generating crossovers by nicking DHJ intermediates. Our results also demonstrate that MEI-9 is not essential for meiotic MMR of base–base mismatches, but may have specialized roles in repair of loops, in repair of products that lack nicks, and in short-patch repair that occurs when the canonical MMR pathway is disrupted. Future studies with different recombination substrates and with mutants that are simultaneously defective in CO formation and canonical MMR, such as *mei-9*; *Msh6* double mutants, can further test these hypotheses and should provide significant new insights into mechanisms of meiotic recombination.

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