The Drosophila Meiotic Recombination Gene *mei-9* Encodes a Homologue of the Yeast Excision Repair Protein Rad1

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ABSTRACT

Meiotic recombination and DNA repair are mediated by overlapping sets of genes. In the yeast *Saccharomyces cerevisiae,* many genes required to repair DNA double-strand breaks are also required for meiotic recombination. In contrast, mutations in genes required for nucleotide excision repair (NER) have no detectable effects on meiotic recombination in *S. cerevisiae.* The *Drosophila melanogaster mei-9* gene is unique among known recombination genes in that it is required for both meiotic recombination and NER. We have analyzed the *mei-9* gene at the molecular level and found that it encodes a homologue of the *S. cerevisiae* excision repair protein Rad1, the probable homologue of mammalian XPF/ERCC4. Hence, the predominant process of meiotic recombination in Drosophila proceeds through a pathway that is at least partially distinct from that of *S. cerevisiae,* in that it requires an NER protein. The biochemical properties of the Rad1 protein allow us to explain the observation that *mei-9* mutants suppress reciprocal exchange without suppressing the frequency of gene conversion.

HOMOLOGOUS recombination is an essential feature of meiosis in many organisms. Recombination ensures the accurate disjunction of homologous chromosomes from one another by allowing the formation of physical linkages (chiasmata) derived from reciprocal exchange events (HAWLEY 1988). The molecular pathway by which recombination occurs is unknown, although a number of attractive models have been proposed (for review, see STAHL 1994). The model proposed by HOLLIDAY (1964) 30 years ago has been particularly influential, contributing two key features that have been incorporated into all subsequent models. The first of these is the creation of heteroduplex DNA, in which each strand of a double-stranded DNA helix is derived from a different parental molecule, as a central component of the recombination process. The existence of heteroduplex DNA has been confirmed by both physical studies (GOYON and LICHTEN 1993; NAG and PETES 1993) and the observation of postmeiotic segregation (PMS) events (WHITE *et al.* 1985). PMS occurs when a mismatch within heteroduplex DNA is not repaired through meiosis and both sequences become fixed in the first postmeiotic round of DNA synthesis. Usually, however, mismatches within heteroduplex are repaired, thereby either restoring the sequence originally on that chromatid or replacing it with the sequence of the homologous chromatid. The latter possibility results in gene conversion, the nonreciprocal transfer of information from one site to another.

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The second important feature of HOLLIDAY's model is the Holliday junction, a chi-shaped DNA structure connecting two parental DNA molecules. Resolution of a Holliday junction occurs when two strands of like polarity are cleaved, and their ends interchanged and religated. Depending on the two strands chosen, resolution can result in a crossover (*i.e.,* the exchange of flanking markers) or a noncrossover. Because proposed recombination intermediates contain one or two Holliday junctions adjacent to or flanking a region of heteroduplex DNA, gene conversion or PMS can be associated with both crossovers and noncrossovers.

Clues to the molecular mechanism of meiotic recombination come from the observation that many of the genes required for this process are also required to repair certain types of DNA damage. In the yeast *Saccharomyces cerevisiae,* a number of meiotic recombination genes are also required to repair DNA double-strand breaks (GAME *et al.* 1980; PRAKASH *et al.* 1980), suggesting models in which recombination is initiated by a double-strand break (SZOSTAK *et al.* 1983). In contrast, mutations in genes required for the nucleotide excision repair (NER) pathway, a versatile system that repairs many types of DNA damage (for review, see HOEIJMAKERS 1993; TANAKA and WOOD 1994; FREIDBERG *et al.* 1995), have no apparent effects on meiotic recombination in *S. cerevisiae* (SNOW 1968; PRAKASH *et al.* 1993).

Many of the genes known to be required for meiotic recombination in *Drosophila melanogaster* are also required in mitotic cells (BAKER *et al.* 1978). Unlike the case in *S. cerevisiae,* however, at least one of these, *mei-9,* is required for nucleotide excision repair (BOYD *et*

al. 1976b; HARRIS and BOYD 1980). Mutations in *mei-9* were first recovered in a screen by BAKER and CARPENTER (1972) for *X*-linked mutations causing high levels of meiotic nondisjunction. Meiotic nondisjunction in females homozygous for *mei-9* mutations results from a decrease in the level of meiotic crossing over to <10% of the normal level. Despite the substantial decrease in reciprocal exchange, meiotic gene conversion occurs at an approximately normal level (ROMANS 1980; CARPENTER 1982). However, *mei-9* females exhibit high levels of PMS, which is manifested in the progeny as individuals who carry a single maternal chromosome but are mosaic for both maternal alleles (ROMANS 1980; CARPENTER 1982). Hence, *mei-9* females are capable of generating recombination intermediates containing heteroduplex DNA but are defective both in the repair of mismatches within the heteroduplex and in the resolution of these intermediates as reciprocal exchanges.

Alleles of *mei-9* have also been recovered in screens for mutations conferring sensitivity to mutagens (BOYD *et al.* 1976a). At least some of the mutagen sensitivity of *mei-9* mutants stems from an absolute block in NER (BOYD *et al.* 1976b; HARRIS and BOYD 1980). The requirement for an NER gene in meiotic exchange is somewhat surprising, given that no meiotic recombination role has been found for any NER gene in *S. cerevisiae*. To understand the role of *mei-9* in meiotic recombination, we analyzed the gene at the molecular level. We found that *mei-9* encodes a homologue of the yeast excision repair protein Rad1, which is not required for meiotic recombination in *S. cerevisiae*.

MATERIALS AND METHODS

Drosophila stocks and culture: Except where noted, genetic markers are described in LINDSLEY and ZIMM (1992) and FLYBASE (1994). Flies were reared on standard cornmeal-molasses-dextrose medium at 25°.

Methyl methanesulfonate treatment: To test for sensitivity to methyl methanesulfonate (MMS), adults were crossed in glass shell vials at 25° for 2 days before being removed. After one additional day, 250 μl 0.08–0.1% MMS (Sigma) in water was added to the medium.

***P*-element construct and transformation:** *P{w⁺ mei-9⁺}* was created by subcloning sequences from the *Eco*RI site in λXIII.62 (PFLUGFELDER *et al.* 1990), which is immediately distal to a genomic *Bam*HI site, to the *Not*I site within the transcription unit proximal to *mei-9*, into pCaSpeR4 (PIRROTTA 1988). Germline transformation was carried out essentially as in RUBIN and SPRADLING (1982).

To test for rescue of MMS sensitivity, single *w; P{w⁺ mei-9⁺}/+* males were mated to three *w mei-9^{AT2}/FM7, B* females. The progeny larvae were treated with MMS as described above, and the number of B^+ w^+ and B^+ w^- males that eclosed were counted.

Meiotic nondisjunction was measured by crossing two to three *w mei-9^{AT2}; P{w⁺ mei-9⁺}* females to $\widehat{XY}$, *In(1)EN, v f B; C(4)RM, ci ey^R* males. The normal progeny of this cross are *w mei-9^{AT2}/$\widehat{XY}$, In(1)EN, v f B* (B females) and *w mei-9^{AT2}/0* (B^+ males). Half of the diplo-*X* ova are recovered as *w mei-9^{AT2}/w mei-9^{AT2}* (B^+ females), whereas the other half die. Similarly, half of the nullo-*X* ova are recovered as *X Y, In(1)EN, v f B/0* (B males), and half die. The *X* nondisjunction (ND) frequency is corrected for the loss of half of the exceptional progeny.

Location of the *mei-9^{RT1} P* element: The position of the *mei-9^{RT1} P*-element was determined by PCR. Reactions contained 10–20 ng genomic DNA, 200 μM each dNTP, 100 pmol of each primer (CGATTGATTGTATCTTCC, corresponding to *mei-9* bases 839–822 on the reverse strand, and CCCGCGGCCGCGACGGGACCACCTTATGTTATTTCATC, which contains the *P* element 31-bp inverted repeat and a *Not*I site, kindly provided by BRIAN CALVI), 1 mM $MgCl_2$, and 2 U Taq DNA polymerase (Promega) in 50 μl of 1× buffer supplied. Reactions were subjected to 25 cycles of 94°, then 55°, then 72° for 1 min each. The resulting fragment, which was amplified from *mei-9^{RT1}* but not from either of two revertants or from either *mei-9^{RT2}* or *mei-9^{RT4}*, was digested with *Pst*I and *Not*I and subcloned into pBlueScript KS+ (Stratagene) for sequencing.

Sequencing and sequence analysis: Double-strand sequencing was done using the Sequenase version 2.0 kit (U.S. Biochemicals), using subclones and gene-specific primers. DNA sequences were assembled and analyzed with the University of Wisconsin Genetics Computing Group (UWGCG) programs (DEVEREUX *et al.* 1984). Searches of the sequence databases were done on the National Center for Biotechnology and Information (NCBI) network server using the Basic Local Alignment Search Tool (BLAST) (ALTSCHUL *et al.* 1990). Protein sequence alignments were generated with the UWGCG programs GAP and PILEUP, using the BLOSUM62 amino acid substitution matrix (HENIKOFF and HENIKOFF 1992).

Genebank accession number: The GenBank accession number for the *mei-9* sequence reported for in this paper is U27181.

RESULTS

***mei-9* is a component of the predominant meiotic recombination pathway:** Mutations in *mei-9* result in a severe decrease in the level of meiotic exchange in *D. melanogaster* females to ~10% of that observed in wild type (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974). The residual crossovers observed could be due to residual activity in the alleles tested, or they may arise through a second meiotic recombination pathway. Two lines of evidence support the second interpretation. First, each of several *mei-9* alleles assayed allows some residual exchange (BAKER and CARPENTER 1972; GRAF *et al.* 1979; BAKER *et al.* 1980). Second, each of several mutant alleles of a second locus, *mei-218*, cause a decrease in the level of meiotic exchange similar to that seen in *mei-9* but also allow some residual exchanges to occur (BAKER and CARPENTER 1972; K. S. MCKIM and R. S. HAWLEY, unpublished data).

The existence of comparable levels of residual exchange in both *mei-9* and *mei-218* mutants suggests that these two genes act in the same meiotic exchange pathway. To test this possibility, we measured exchange in females simultaneously mutant for both genes. The level of residual exchange in doubly mutant females is comparable to the level seen in either single mutant

TABLE 1

Meiotic exchange in *mei-9 mei-218* females

Genotype	Exchange within the interval map units					n
	al-dp	*dp-b*	*b-pr*	*pr-cn*	*al-cn*	
mei-9^a *mei-218*/+	10.4	25.8	4.8	0.92	41.8	1517
mei-9^a *mei-218*/*mei-9*^{a}	0.94 (9.0)	3.5 (14)	1.1 (23)	0.12 (13)	5.7 (11)	1687
mei-9^a *mei-218*/*mei-218*	0.58 (5.5)	1.2 (4.8)	0.50 (10)	0.31 (33)	2.6 (6.1)	2597
mei-9^a *mei-218*	0.40 (3.9)	1.3 (4.9)	0.68 (14)	0.61 (66)	3.0 (7.1)	2924

Values in parentheses are percent of control.

(Table 1). Based on this observation, we propose that *mei-9* and *mei-218* define a single pathway for meiotic exchange, and that this is the predominant meiotic exchange pathway in Drosophila. In the absence of either *mei-9* or *mei-218*, an alternative pathway can provide a low level of exchange. We argue that it is this secondary pathway, and not some residual *mei-9* or *mei-218* activity, that allows the low levels of recombination observed in females homozygous for either or both of these mutants.

The data in Table 1 also allow us to order *mei-9* and *mei-218* within the exchange pathway. Although mutations in either gene decrease meiotic exchange to <10% of wild-type levels, the pattern of residual exchanges differs between these genes (BAKER and CARPENTER 1972). In *mei-218* females, exchange is decreased less in proximal intervals than in other regions. The result is that the residual exchanges occur with a distribution more proportional to physical length than do exchanges in wild-type females. In contrast, exchange in *mei-9* is decreased uniformly throughout all intervals, so that the distribution of residual exchanges parallels that of exchange in wild type. The distribution of residual exchanges in the *mei-9 mei-218* females resembles that of *mei-218* females (Table 1). Therefore, *mei-218* is epistatic to *mei-9* with respect to this phenotype, suggesting that *mei-218* acts earlier than *mei-9* in the exchange pathway. It is also possible that *mei-218* is required for two separate events, one of which establishes the normal distribution of exchanges.

To better understand the recombination pathway defined by *mei-9* and *mei-218*, we set out to analyze these genes at the molecular level. We describe here the molecular cloning of *mei-9*.

Molecular cloning of the *mei-9* locus: The *mei-9* gene was previously localized to region 4B3–4 to 4C1 on the standard polytene chromosome map (BANGA *et al.* 1986). Three existing *mei-9* alleles were recovered in a screen in which *P*-transposable elements were mobilized, and *in situ* hybridization to polytene chromosomes showed that one allele, *mei-9*^{RT1}, had *P* sequences in region 4B-C (YAMAMOTO *et al.* 1990). To confirm that *mei-9*^{RT1} contains a *P* element inserted into *mei-9*, we used a genetic source of P transposase to generate revertants of *mei-9*^{RT1}. Females of the genotype *mei-9*^{RT1}/*FM7, B; TMS, Sb P*[Δ*2–3*](99B)/+ were crossed to *mei-9*^{a} males. The resulting larvae were treated with MMS, and surviving non-Bar adults were retested to distinguish between *mei-9*^{RT1} escapers and revertants. The recovery of three independent revertants confirmed that the *mei-9*^{RT1} allele is indeed caused by the insertion of a *P* element, providing an avenue for cloning the locus.

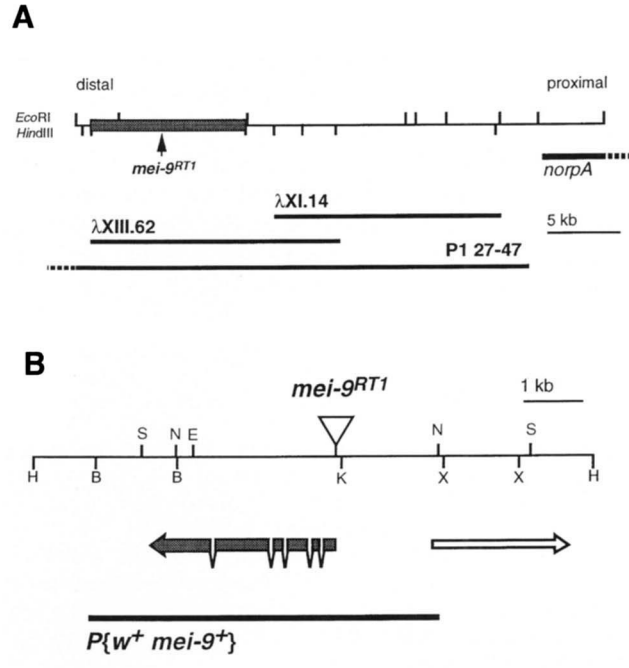

FIGURE 1.—Physical map of the *mei-9* region. (A) Restriction map of the *mei-9* to *norpA* genomic region. Tick marks represent *Eco*RI (above) and *Hin*dIII (below) sites. The distal end of *norpA* is indicated. The approximate regions contained in two lambda clones (PFLUGFELDER *et al.* 1990) and a P1 clone (SMOLLER *et al.* 1991) are shown below. *mei-9* was initially mapped to the distal 10-kb *Hin*dIII fragment (bold line) because of an additional *Hin*dIII site in *mei-9*^{RT1} but not in revertants. (B) A restriction map of the 10-kb *Hin*dIII fragment containing the *mei-9*^{RT1} *P* element. The position of the *P* element in *mei-9*^{RT1} is indicated by ▽. Two divergently transcribed ovarian transcripts are derived from this region: a distal 3.4-kb *mei-9* transcript and a proximal 2.3-kb transcript of unknown function. ■ in the *mei-9* transcript represent exons. The line at the bottom indicates the extent of the genomic fragment that rescues *mei-9* mutant phenotypes. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, *Not*I; S, *Sal*I; X, *Xba*I.

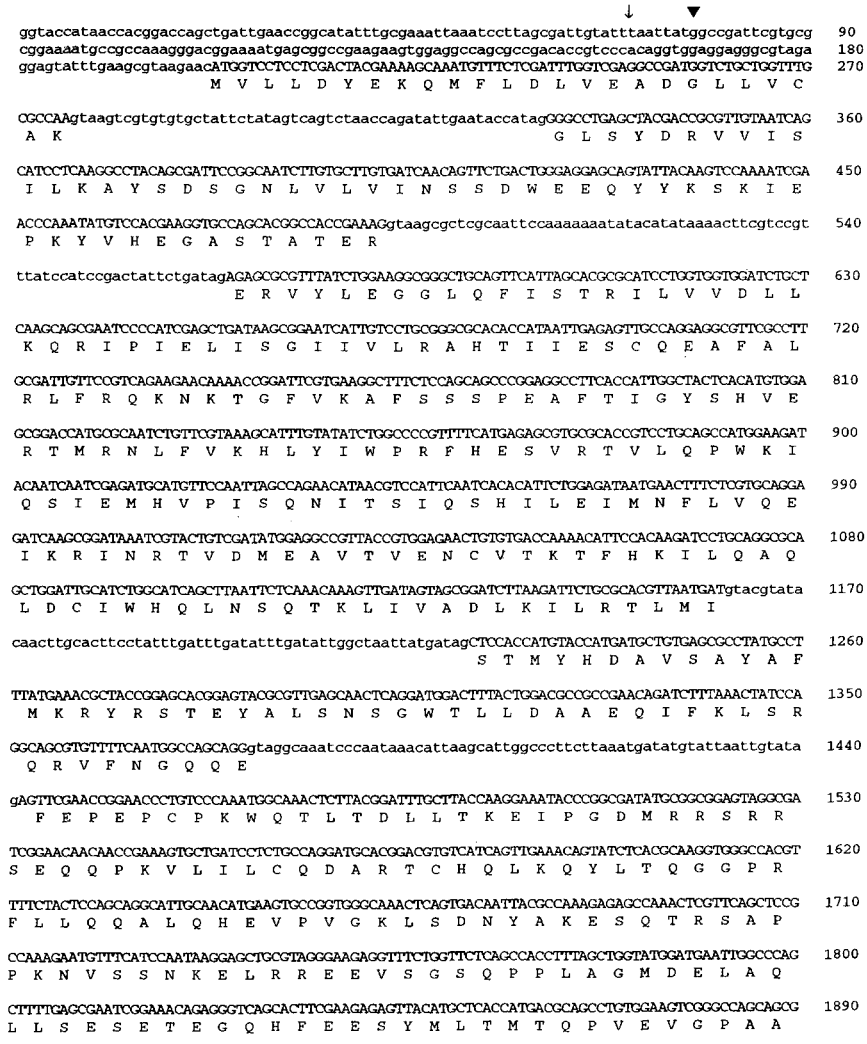

FIGURE 2.—*mei-9* genomic sequence and predicted MEI-9 amino acid sequence. The sequence from the *Kpn*I site to the distal *Sal*I site (Figure 1) is shown, with coding sequences in uppercase above a conceptual translation. Both genomic DNA and a *mei-9* cDNA were sequenced. The arrow at base 67 indicates the first base of the *mei-9* cDNA. Because the cDNA library was constructed with *Not*I sites at the 3′ end (STROUMBAKIS and TOLIAS 1994), the cDNA clone terminates at the internal *Not*I site at position 2922 (underlined), 370 bp from the termination codon. ▼ indicates the position of the *P* element responsible for the $mei\text{-}9^{RT1}$ mutation.

mei-9 is just distal to the previously cloned gene *norpA* (BANGA *et al.* 1986). We obtained clones extending distally from *norpA* (PFLUGFELDER *et al.* 1990) and used them to probe blots of DNA from wild type, $mei\text{-}9^{RT1}$, and revertants of $mei\text{-}9^{RT1}$. We found a *P*-element insertion that is present in $mei\text{-}9^{RT1}$ but not in either of two independent revertants of $mei\text{-}9^{RT1}$ (data not shown). This insertion maps about 20 kb distal to the 5′ end of *norpA* (Figure 1).

A 6-kb genomic fragment contains meiotic and mitotic *mei-9* activity: By Northern blot analysis of RNA from either ovaries or whole females, we identified two transcripts near the $mei\text{-}9^{RT1}$ *P*-element insertion point, a proximal transcript of 2.3 kb and a distal transcript of 3.4 kb (Figure 1). We screened a cDNA library prepared from ovarian mRNA (STROUMBAKIS and TOLIAS 1994) and obtained 10 cDNAs corresponding to the 2.3-kb transcript and one corresponding to the 3.4-kb transcript. The $mei\text{-}9^{RT1}$ *P* element is inserted into sequences corresponding to the 5′ untranslated region of the distal 3.4-kb transcript (Figure 2), making this the likely candidate for the *mei-9* gene.

To confirm that the 3.4-kb transcript indeed corresponds to *mei-9*, we subcloned the genomic region encompassing it into a *P*-element vector (Figure 1) and obtained germline transformants. We tested several independent insertions of $P\{w^{+}\ mei\text{-}9^{+}\}$ for the ability to rescue the MMS sensitivity of *mei-9*. Each of 10 insertion lines tested fully rescued this phenotype (Table 2). We further tested some lines for the ability to rescue the meiotic nondisjunction phenotype of *mei-9* females and found that each fully rescued this phenotype as well (Table 3). We conclude that *mei-9* meiotic and mitotic activity is fully contained within a 6-kb fragment containing the 3.4-kb transcription unit.

***mei-9* encodes a homologue of the yeast excision repair protein Rad1:** We sequenced the *mei-9* cDNA and the genomic region encompassing it (Figure 2). Conceptual translation of this sequence predicts a MEI-9 protein of 946 amino acid residues. A BLAST search (ALTSCHUL *et al.* 1990) of the sequence databases revealed that the predicted MEI-9 protein is closely related to the excision repair proteins Rad1 from *S. cerevisiae* and Rad16 from *Schizosaccharomyces pombe* (Figure

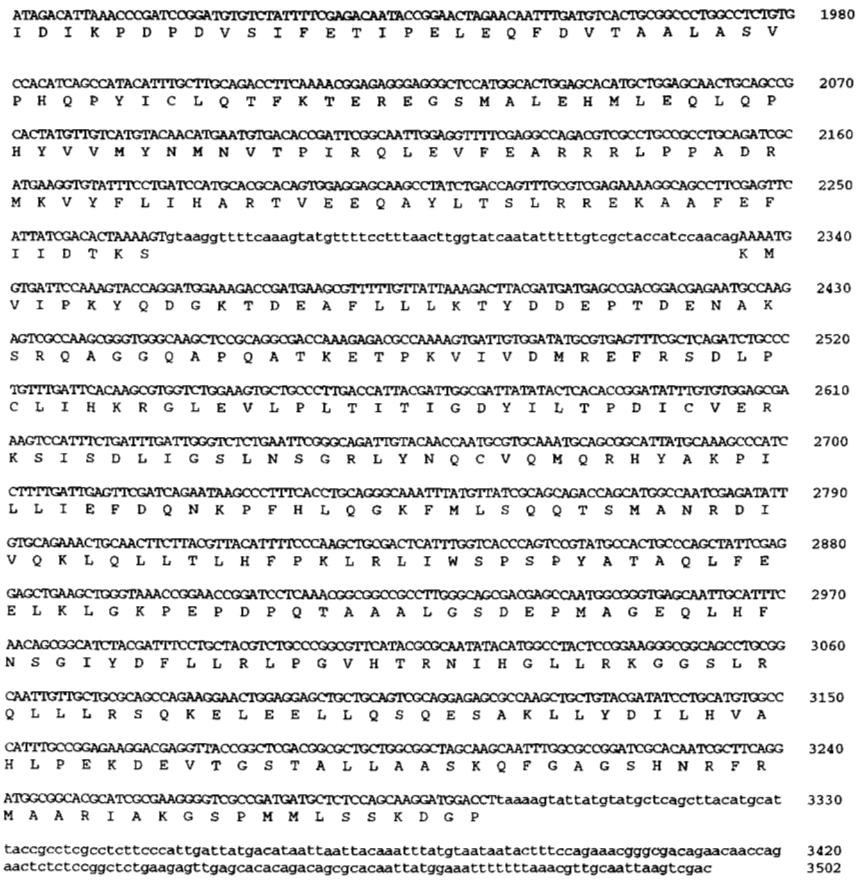

FIGURE 2.—*Continued*

3). Overall, there is 30% identity and 51% similarity between MEI-9 and Rad1, and 40% identity and 58% similarity between MEI-9 and Rad16. Three of the five *mei-9* introns (the second, third, and fifth) are in positions identical to those of three of seven *rad16* introns (the first, third, and sixth, respectively) (Figure 2).

DISCUSSION

We have shown that *mei-9* encodes the Drosophila homologue of *S. cerevisiae* Rad1 and *S. pombe* Rad16. Like *mei-9*, both *RAD1* (WILCOX and PRAKASH 1981) and *rad16* (SCHMIDT *et al.* 1987) are required for NER. These proteins are believed to be the yeast homologues of human xeroderma pigmentosum complementation group F (XPF) and the mammalian excision repair protein ERCC4 (BIGGERSTAFF *et al.* 1993; VAN VUUREN et al. 1993). Genetic characterization of *RAD1* and *rad16* has shown these genes to have additional mitotic functions besides their roles in NER. When a double-strand break occurs within a region of nonhomology between repeats, *RAD1* is required to remove the nonhomologous ends to allow repair through a putative single-strand annealing mechanism (FISHMAN-LOBELL and HABER 1992). *RAD1* also defines a *RAD52*-independent pathway for mitotic intrachromosomal repeat recombination that occurs through an undetermined pathway or pathways (KLEIN 1988; SCHIESTL and PRAKASH 1988). The *S. pombe* homologue, *rad16*, is also required during mating type switching (SCHMIDT *et al.* 1989).

TABLE 2

Rescue of mutagen sensitivity by $P\{w^+, mei\text{-}9^+\}$

	no MMS		+ MMS	
P Line	w males	$P\{w^+, mei\text{-}9^+\}$ males	w males	$P\{w^+, mei\text{-}9^+\}$ males
1	69	64	0	65
2	26	24	0	32
3	32	26	1	50
4-10[a]	296	274	0	225

[a] Summed totals for seven additional insertion lines.

An important clue to understanding the multiple genetic functions of *RAD1* and *rad16* came from the discovery that Rad1, together with Rad10, the homologue of the mammalian excision repair protein ERCC1 (VAN DUIN *et al.* 1986) and *S. pombe* Swi10 (RODEL *et al.* 1992), constitutes a single-strand DNA endonuclease *in vitro* (TOMKINSON *et al.* 1993). In NER, the Rad1/Rad10 complex is believed to make an incision 5′ to the site of DNA damage, allowing removal of a section of the damaged strand (BARDWELL *et al.* 1994). MEI-9 presumably plays an analogous role in NER in Drosophila. In the single-

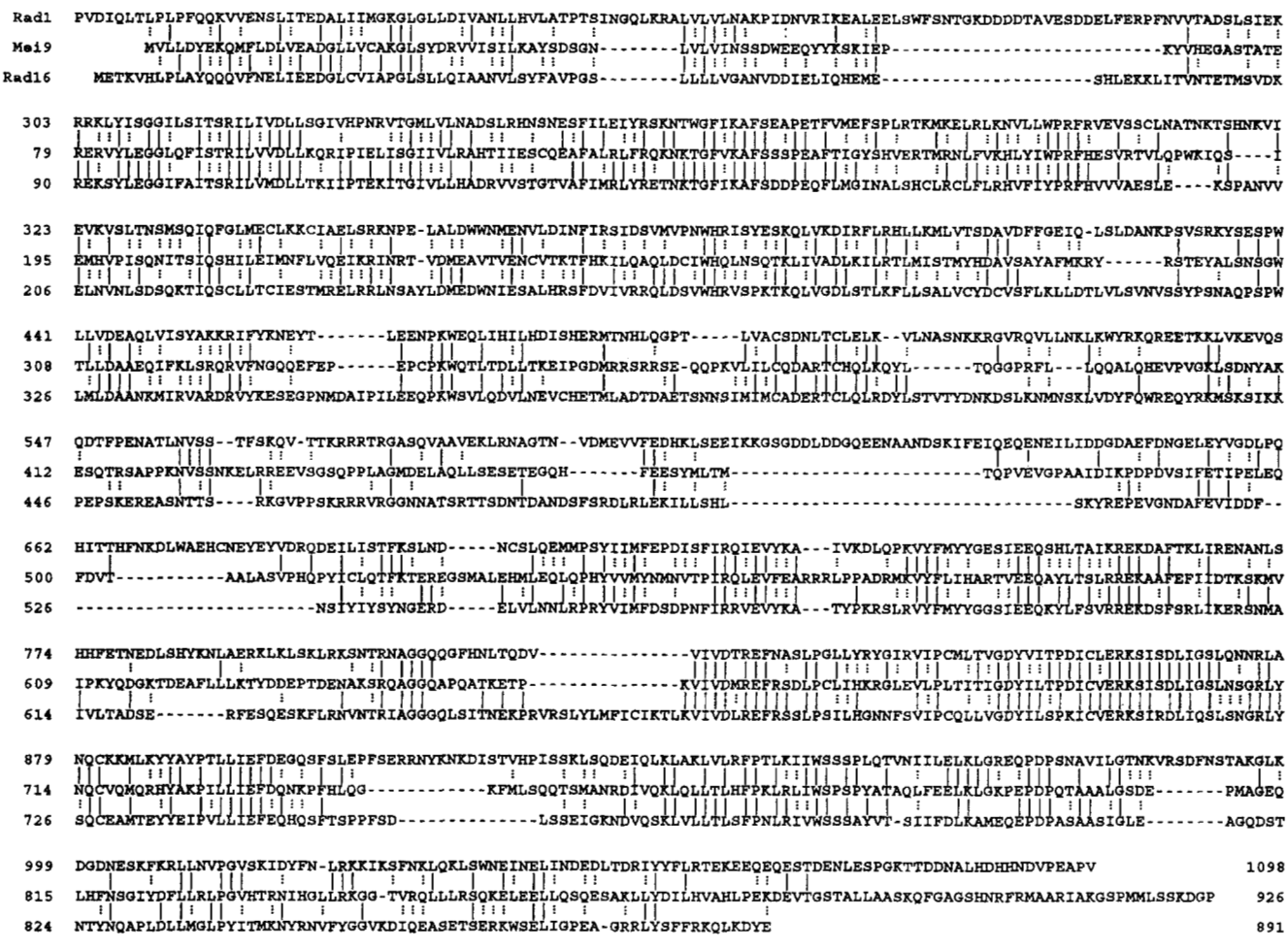

FIGURE 3.—Comparison of *D. melanogaster* MEI-9 with *S. cerevisiae* Rad1 and *S. pombe* Rad16. The predicted amino acid sequences of MEI-9, Rad1, and Rad16 are shown in a best-fit alignment to one another, with dashes indicating gaps introduced to give the best alignment. Residues in Rad1 or Rad16 that are identical to the corresponding residue of MEI-9 are indicated with a solid line, whereas similar residues are indicated with a dotted line. Similarity is defined as residue pairs that score positive with the BLOSUM62 substitution matrix (HENIKOFF and HENIKOFF 1992).

strand annealing pathway, the Rad1/Rad10 endonuclease is thought to cut immediately 5′ to the boundary of the single strand-double strand junction of a splayed DNA structure, allowing removal of the nonhomologous single strand (BARDWELL *et al.* 1994). *RAD1/RAD10*-mediated *RAD52*-independent mitotic recombination may occur through either the single-strand annealing pathway or a pathway in which Rad1/Rad10 mediates Holliday junction resolution (HABRAKEN *et al.* 1994). In *S. pombe* mating type switching, Rad16 and Swi10 are believed to resolve some unknown intermediate (SCHMIDT *et al.* 1987), a function that can be readily explained in terms of an endonuclease activity.

What is the function of MEI-9 in meiotic recombination? In *mei-9* females, meiotic recombination is strongly suppressed (BAKER and CARPENTER 1972), but the frequency of gene conversion is normal or perhaps slightly increased (ROMANS 1980; CARPENTER 1982). The finding that *mei-9* encodes the homologue of a protein that cleaves some types of DNA junctions *in vitro* suggests

TABLE 3

Rescue of *mei-9* meiotic defects by *P*{w$^+$, mei-9$^+$}

P Line	B females	B$^+$ males	B$^+$ females	B males	*X* nondisjunction (%)
None	316	356	84	85	33
1	194	157	0	0	0
2	520	523	0	1	0.2
3	331	360	0	0	0

that the MEI-9 protein may act directly to resolve Holliday junctions within recombination intermediates, a role clearly consistent with the exchange defect seen in *mei-9* females. An *in vitro* Holliday junction resolving activity has been reported for Rad1 (HABRAKEN *et al.* 1994), although the interpretation of this result has been questioned by WEST (1995), in part because of the lack of an effect of *rad1* mutations on meiotic exchange.

Because both exchange and gene conversion without exchange have been presumed to require nicking of two strands at the Holliday junction, followed by exchange and religation with one another (HOLLIDAY 1964; MESELSON and RADDING 1975; SZOSTAK *et al.* 1983), a simple defect in Holliday junction resolution would seem to fail to explain the decrease in flanking marker exchange without a corresponding decrease in gene conversion that is observed in *mei-9*. The resolution to this conundrum may lie in the suggestion by THALER *et al.* (1987) (see also STAHL 1994) that at least the double Holliday junction-recombination intermediates observed in yeast (SCHWACHA and KLECKNER 1994) can be resolved without strand nicking, exchange, and religation, simply by topoisomerase activity, yielding either gene conversion, restoration, or possible PMS. We suppose that mutants like *mei-9*, which are conversion-proficient but exchange-defective, lack only the strand cleavage and ligation pathway that gives rise to flanking marker exchange.

Females homozygous for *mei-9* mutations are also defective in the repair of mismatches within meiotic heteroduplex DNA (ROMANS 1980; CARPENTER 1982). This may represent a second, independent requirement for MEI-9, perhaps related to its excision repair function. One argument against heteroduplex mismatch repair being mediated by the standard NER pathway is that meiotic gene conversion tracts in Drosophila are continuous for several hundred bp (CURTIS *et al.* 1989), whereas NER tracts are, at least in mammalian cells, only about 30 bp long (CLEAVER 1994). If the excision repair pathway does mediate heteroduplex repair in Drosophila, this would be in contrast to the case in *S. cerevisiae*, where NER genes, including *RAD1*, are not required for meiotic heteroduplex mismatch repair (DICAPRIO and HASTINGS 1976). Rather, repair of such mismatches in *S. cerevisiae* requires genes belonging to the mismatch repair pathway (WILLIAMSON *et al.* 1985; KRAMER *et al.* 1989; ALANI *et al.* 1994). The contribution of this pathway to meiotic heteroduplex repair in Drosophila is unknown.

We have proposed that MEI-9 carries out two independent functions during meiotic recombination. An alternative is that MEI-9 is required for a single step that must occur both to allow the repair of mismatches and to allow resolution as a crossover. AGUILERA and KLEIN (1989) found that in their intrachromosomal mitotic recombination assay, long gene conversion tracts were preferentially associated with crossovers. In this assay, *rad1* mutations resulted in a reduced recovery of long conversion tracts, leading to a reduced crossover rate (although crossing over was also reduced even in the long conversion tracts observed in *rad1* mutants). This suggests either a Rad1-mediated long conversion tract pathway or a role of Rad1 in heteroduplex rejection. In heteroduplex rejection models, mismatch repair proteins prevent the extension of heteroduplex in response to bp mismatches, thereby preventing exchange, but not repair or gene conversion, between homeologous sequences (RAYSSIGUIER *et al.* 1989; ALANI *et al.* 1994). An argument against a role of *mei-9* in a heteroduplex rejection model is that gene conversion tracts from *mei-9* females are similar in length to those produced by wild-type females (CURTIS and BENDER 1991). In addition, CURTIS *et al.* (1989) found that the conversion tracts they sequenced that were associated with a crossover were shorter than simple conversion tracts they sequenced.

In contrast to *mei-9*, *rad1* mutations do not affect the level of meiotic exchange (SNOW 1968). [mutations in *rad16* do not affect the level of meiotic gene conversion (SCHMIDT *et al.* 1987); however, their effects on meiotic exchange have not been reported.] The finding that the meiotic recombination gene *mei-9* encodes the homologue of a gene not required for meiotic recombination in *S. cerevisiae* reveals differences between the yeast and fly meiotic recombination pathways. We do not know whether MEI-9 carries out a function in Drosophila meiotic recombination that is mediated by some other protein in Saccharomyces, or whether Drosophila and Saccharomyces use fundamentally different recombination pathways. Our data on residual exchange in *mei-9 mei-218* double mutants suggests the existence of a secondary exchange pathway in Drosophila. One intriguing possibility is that the predominant meiotic recombination pathway in yeast, the *RAD52*-dependent pathway, is used in this minor role as a backup pathway in Drosophila.

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