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Drosophila MUS312 Interacts with the Nucleotide Excision Repair Endonuclease MEI-9 to Generate Meiotic Crossovers

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Summary

MEI-9 is the *Drosophila* homolog of the human structure-specific DNA endonuclease XPF. Like XPF, MEI-9 functions in nucleotide excision repair and interstrand crosslink repair. MEI-9 is also required to generate meiotic crossovers, in a function thought to be associated with resolution of Holliday junction intermediates. We report here the identification of MUS312, a protein that physically interacts with MEI-9. We show that mutations in *mus312* elicit a meiotic phenotype identical to that of *mei-9* mutants. A missense mutation in *mei-9* that disrupts the MEI-9–MUS312 interaction abolishes the meiotic function of *mei-9* but does not affect the DNA repair functions of *mei-9*. We propose that MUS312 facilitates resolution of meiotic Holliday junction intermediates by MEI-9.

Introduction

Genetic recombination is essential for the maintenance of genome stability. In meiosis, accurate segregation of homologous chromosomes depends on generation of crossovers by a homologous recombination pathway (reviewed in Kleckner, 1996; Roeder, 1997). Recombination is also involved in the repair of some types of DNA damage, including double-strand breaks (DSBs) (reviewed in Haber, 2000b) and interstrand crosslinks (ICLs) (reviewed in McHugh et al., 2001). These recombination pathways are carried out by overlapping sets of genes. For example, some of the genes that carry out meiotic recombination are also involved in recombinational repair of DSBs (Haber, 2000a). An example of this crossfunctionality is provided by the *Drosophila melanogaster mei-9* gene, which is required for meiotic recombination, ICL repair, and nucleotide excision repair (NER) (Baker and Carpenter, 1972; Boyd et al., 1976b; Sekelsky et al., 1995).

The meiotic recombination defect in *mei-9* includes a 90%–95% decrease in crossovers (Baker and Carpenter, 1972). Most *Drosophila* meiotic recombination mutants affect both the number and position of crossovers; *mei-9* is unique in that only the number of crossovers is affected. Baker and Carpenter (1972) interpreted this to mean that *mei-9* is required very late in the meiotic crossover pathway, in the actual process of generating crossovers. According to current molecular models of meiotic recombination, the final step in generating crossovers is the resolution of an intermediate that contains two Holliday junctions (reviewed in Stahl, 1996). Genetic analysis therefore argues that *mei-9* functions in the resolution of this intermediate.

The MEI-9 protein is the homolog of the human and yeast NER proteins XPF and Rad1p, respectively (Sekelsky et al., 1995; Sijbers et al., 1996). These proteins share a highly conserved nuclease domain (Aravind et al., 1999). A notable feature of this protein family is that they each form a heterodimer with a smaller noncatalytic subunit, Ercc1 in mammals and Rad10p in yeast (Bardwell et al., 1992; Park and Sancar, 1994). In vitro nuclease activity of XPF and Rad1p requires the presence of these noncatalytic subunits (Bardwell et al., 1994; Davies et al., 1995; Park et al., 1995), and genetic analysis confirms that both subunits are also required for the known functions in vivo (Ivanov and Haber, 1995; Schiestl and Prakash, 1990).

Missense mutations in the nuclease domain of MEI-9 abolish its function in both NER and meiotic recombination (Ö.Y. and J.J.S., unpublished data). It therefore seems likely that the meiotic function of MEI-9 involves structure-specific endonuclease activity. Because *mei-9* mutants are defective in the generation of crossovers from an intermediate that contains Holliday junctions, we have proposed that MEI-9 acts directly to resolve these Holliday junctions (Sekelsky et al., 1998, 1995). Although there is no direct evidence for this activity, a recently identified *S. pombe* complex that cuts Holliday junctions has a catalytic subunit, Mus81, whose nuclease domain is related to that of MEI-9 and its homologs (Interthal and Heyer, 2000; Boddy et al., 2001).

The function of MEI-9 in ICL repair is less clear. Although pathways for ICL repair have not been elucidated, it is believed that the major repair pathway involves a double-strand break intermediate that is repaired by homologous recombination (reviewed in McHugh et al., 2001). MEI-9 may function early in the ICL repair pathway to generate the double-strand break intermediate. Alternatively, or in addition, MEI-9 may be involved in the homologous recombination component of the repair pathway. It is possible that this latter process proceeds through a Holliday junction intermediate, and thus the function of MEI-9 in ICL repair may mimic its proposed function in meiotic recombination.

In NER, XPF–Ercc1 makes a single nick on one strand of a partially unwound DNA duplex (Bardwell et al., 1992; Park and Sancar, 1994). Resolution of a four-stranded Holliday junction, however, requires that two opposing nicks be made symmetrically. If the function of MEI-9 is to cut Holliday junctions in meiotic recombination (and possibly ICL repair) intermediates, then it is likely that this activity requires partners either in addition to or in place of ERCC1. Indeed, the Holliday junction cleavage activity of *S. pombe* Mus81 requires a second subunit (Eme1) as well as additional unidentified proteins (Boddy et al., 2001). Furthermore, when the *S. cerevisiae* homologs, Mus81p and Mms4p, are coexpressed in bacteria and purified, they cut branched DNA substrates but not Holliday junctions (Kaliraman et al., 2001). One explana-

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Table 1. Meiotic Crossing Over in *mei-9* and *mus312* Mutants

Genotype	Exchange within the Interval (map units)				Total Map Distance	n
	net-dpp	dpp-dp	dp-b	b-pr		
wild-type[a]	4.0	9.0	35.0	6.0	54.0	
mei-9^{A2}	0.42	0.80	5.66	0.58	7.46	1872
mus312^{D1}	0.27	0.67	2.56	0.67	4.17	741
mus312^{Z1973}	0	0.28	3.06	0.56	3.90	359

[a] Standard map distances from Lindsley and Zimm (1992).

tion is that these preparations lack an accessory protein necessary for Holliday junction cleavage activity.

We took two approaches to identify proteins that may allow MEI-9 to cut Holliday junctions during meiotic recombination. First, we surveyed known mutants for any with a phenotype similar to that of *mei-9* mutants. Second, to identify proteins that interact physically with MEI-9, we conducted a yeast two-hybrid screen using full-length MEI-9 as bait. These two approaches led independently to the identification of the DNA repair gene *mus312*. We demonstrate here that *mus312* encodes a novel protein that must physically interact with MEI-9 to generate meiotic crossovers. We also show that MUS312 is involved in the repair of DNA interstrand crosslinks but is at least partially independent of MEI-9 in repairing this type of damage.

Results and Discussion

Formation of Meiotic Crossovers Requires *mus312*

We surveyed known meiotic mutants for phenotypes like that of *mei-9* mutants. Green (1981) suggested that *mus312*^{D1} mutants have a meiotic recombination phenotype similar to *mei-9* mutants. We tested this proposal by comparing the meiotic crossover frequencies between *mei-9* and *mus312* mutants. We measured crossing over between *net* and *pr*, a 54-map unit interval spanning the entire euchromatic portion of the left arm of chromosome 2 (Table 1). Both *mei-9*^{A2} (a null allele) and *mus312*^{D1} resulted in a severe decrease in crossing over across the entire chromosome arm. Furthermore, the magnitude of the effect was similar in each interval examined, a phenotype previously described only for *mei-9* mutants.

The *mus312*^{D1} mutation was originally isolated on the basis of hypersensitivity to the DNA crosslinking agent nitrogen mustard (HN2) (Boyd et al., 1981). We obtained two new alleles, *mus312*^{Z1973} and *mus312*^{Z3997}, which were also isolated on the basis of hypersensitivity to HN2 (see Experimental Procedures). Both alleles fail to complement *mus312*^{D1} for this hypersensitivity (Figure 1B and data not shown). We measured X chromosome nondisjunction in females carrying different combinations of *mus312* alleles. Defects in meiotic recombination result in high rates of meiotic chromosome nondisjunction, and this assay is therefore an indirect indicator of recombination defects. All *mus312* mutant genotypes tested resulted in a greater than 70-fold elevation in X chromosome nondisjunction (Table 2 and data not shown). We also directly measured crossing over in *mus312*^{Z1973} mutants, as described above, and found a similar level and distribution to that seen for *mei-9* mutants and for *mus312*^{D1} (Table 1). Thus, three different *mus312* mutations cause both a meiotic defect and a deficiency in the repair of ICLs. We conclude that both phenotypes are associated with mutations in a single gene. Therefore, *mus312* joins *mei-9* as the second member of a class of genes whose products function in the resolution of meiotic recombination intermediates into crossovers.

Interstrand Crosslink Repair Requires *mus312*, but Nucleotide Excision Repair Does Not

Yeast and mammalian homologs of MEI-9 (Rad1p and XPF) function in nucleotide excision repair (NER) as heterodimers with a smaller subunit, Rad10p and Ercc1, respectively (Bardwell et al., 1992; Park et al., 1995). In *S. cerevisiae*, mutations in either *RAD1* or *RAD10* result in an identical phenotype both in NER and in other pathways (Ivanov and Haber, 1995; Schiestl and Prakash, 1990). In *Drosophila*, a protein with sequence similarity to Ercc1 and Rad10p maps to chromosome 2, a different chromosome from *mus312* (Sekelsky et al., 2000). Since there are no mutations in *Ercc1* currently available, it is formally possible, however, that *mus312* functionally substitutes for *Ercc1* in *Drosophila*. To test this possibility, we measured sensitivity of *mus312* mutants to ultraviolet radiation (UV). NER is the primary pathway for removal of UV-induced DNA damage; consequently, mutations in *mei-9* or other NER genes result in extreme hypersensitivity to UV (Figure 1A). We did not detect UV hypersensitivity in *mus312* mutants (Figure 1A), indicating that *mus312* is not essential for NER in *Drosophila*. MUS312 therefore does not substitute for ERCC1 in this pathway.

Both *mei-9* and *mus312* mutants are extremely sensitive to HN2 (Figure 1B). HN2 is a bifunctional alkylating agent capable of introducing both ICLs and mono-adducts (reviewed in Friedberg et al., 1995). Because *mus312* mutants are only weakly sensitive to the monofunctional alkylating agent methyl methanesulfonate (MMS; Figure 1C), we conclude that *mus312* is specifically required in the repair of ICLs.

In contrast, *mei-9* mutants are hypersensitive to both HN2 and MMS (Figures 1B and 1C). Other NER mutations also cause hypersensitivity to MMS, presumably because at least some mono-adducts are repaired by NER (Sekelsky et al., 2000). It is possible that the sensitivity of *mei-9* mutants to HN2 is due to the involvement of NER in removing the mono-adducts induced by this agent. However, mutations in *mus210*, which encodes the *Drosophila* homolog of the NER damage recognition protein XPC (Sekelsky et al., 2000), are only weakly sensitive to HN2 (Henderson et al., 1987). This argues that at least a portion of the *mei-9* hypersensitivity to this

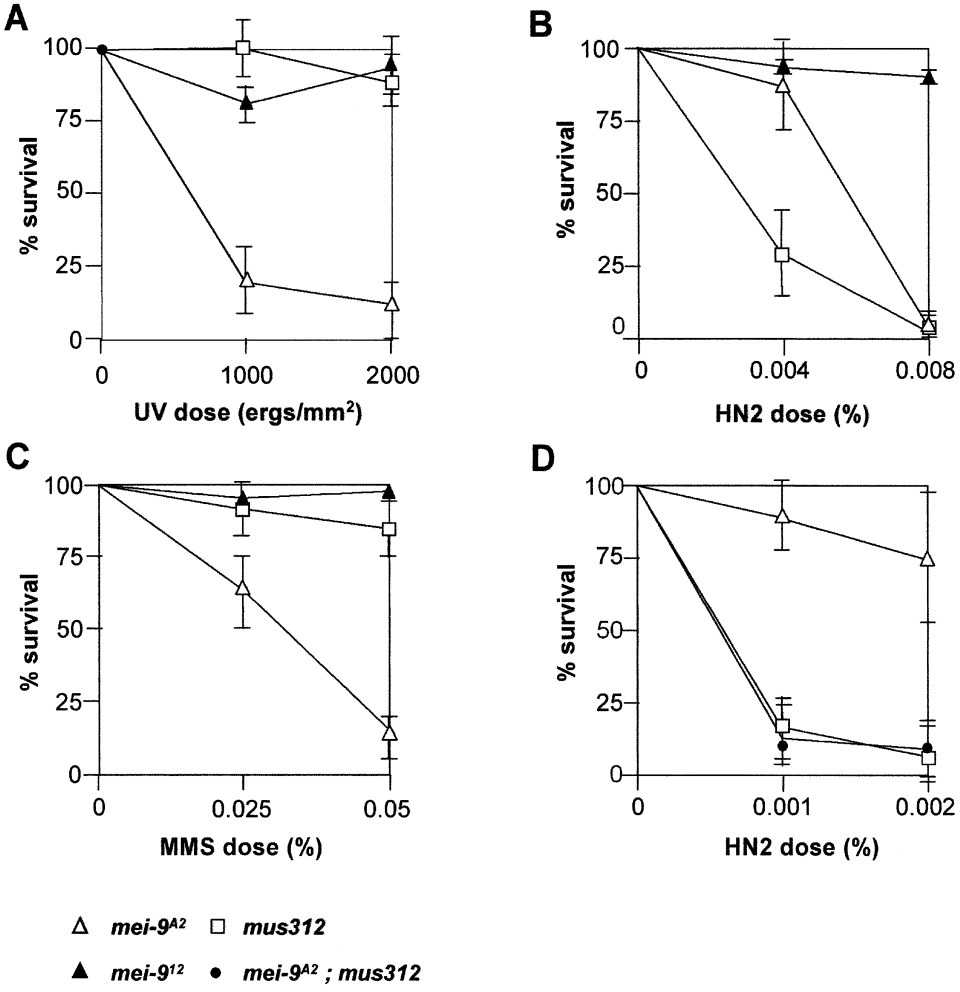

Figure 1. Survival of *mei-9* and *mus312* Mutants after Treatment with DNA Damaging Agents

Percent survival is given relative to wild-type controls (heterozygous siblings; see Experimental Procedures for details). Bars indicate standard deviation of at least two independent experiments. The allele *mei-9*^{A2} is a protein and genetic null, whereas *mei-9*^{12} is a separation-of-function allele (see text); *mus312* experiments were done with *mus312*^{D1}/*mus312*^{Z1973} compound heterozygotes (panels A–C) or *mus312*^{Z1973} homozygotes (panel D). (A) UV irradiation. (B and D) Nitrogen mustard added to the medium. (C) Methyl methanesulfonate added to the medium.

agent is due to defects in ICL repair. Thus, both MEI-9 and MUS312 are involved in ICL repair.

Although both MEI-9 and MUS312 are important in ICL repair, *mus312* mutants are more sensitive to HN2 than are *mei-9* mutants (Figure 1B). It is possible that the MEI-9 function can be partially substituted by another protein in ICL repair, but MUS312 function cannot. Alternatively, *mei-9* and *mus312* may function in separate pathways for the removal of ICLs. To distinguish between these alternatives, we examined HN2 sensitivity in *mei-9 mus312* double mutants. If these two genes are required in separate pathways, the double mutants, which would be defective in both pathways, should be more sensitive than either single mutant. However, we found that *mei-9 mus312* double mutants are identical in sensitivity to *mus312* single mutants (Figure 1D). Our interpretation of this result is that MEI-9 is required for a subset of MUS312-dependent repair of ICLs. The higher sensitivity of *mus312* mutants also reveals that there are MEI-9-independent functions for MUS312.

Table 2. X Chromosome Nondisjunction in *mei-9* and *mus312* Mutants

Genotype	Progeny			X nondisjunction (%)
	normal	nullo-X	diplo-X	
wild-type	3594	2	4	0.33
mei-9^{A2}/*mei-9*^{A2}	857	111	145	37
mus312^{Z1973}/*mus312*^{D1}	1402	150	75	24
mus312^{Z3997}/*mus312*^{D1}	348	38	18	24

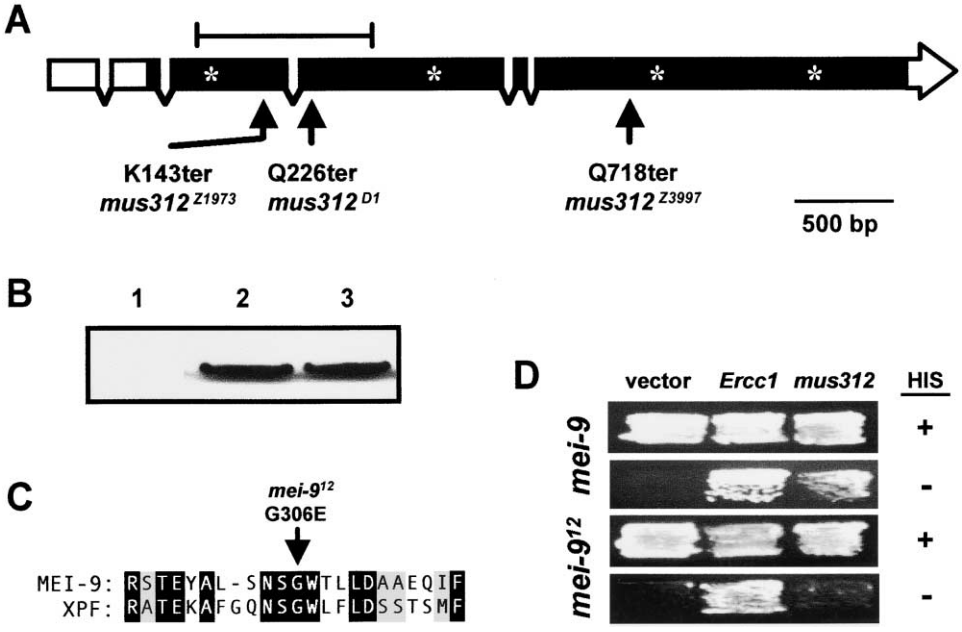

Figure 2. Molecular Map of *mus312* and Interaction between MUS312 and MEI-9

(A) A schematic of the genomic architecture of *mus312* (*CG8601*) is shown. Each box represents an exon, and filled regions designate protein coding sequences. The bar above the map indicates the region contained in the yeast two hybrid clone that interacts with MEI-9 (without the intron). Asterisks represent potential nuclear localization signals.

(B) Interaction between MUS312 and MEI-9 in nuclear extracts (see Experimental Procedures for details). Lane 1 is GST alone; lanes 2 and 3 are GST-MUS312$^{1-387}$. The sample in lane 3 was treated with DNaseI prior to washing.

(C) Sequence of *mei-9^{12}* allele. The sequence surrounding the *mei-9^{12}* substitution is shown for *Drosophila* MEI-9 (residues 296 through 317) and human XPF (residues 306 through 328).

(D) Interaction between MUS312, MEI-9, and MEI-9^{12} in a yeast two hybrid system. Colonies containing pGBD:MEI-9 (upper) or pGBD:MEI-9^{12} (lower) and pACT2 vector, pGAD10:ERCC1, or pGAD10:MUS312 were streaked onto medium lacking threonine and leucine (to select for the presence of the two plasmids) and containing (+) or lacking (−) histidine. Growth on medium lacking histidine is indicative of an interaction between the fusion proteins. The MEI-9, MEI-9^{12}, and ERCC1 fusions were full-length; the MUS312 fusion contained the interaction domain mapping to residues 61–303.

MUS312 Is a Novel Protein that Interacts Physically with MEI-9

Because *mus312* appears to be required in the resolution of meiotic recombination intermediates, we sought to characterize the gene molecularly. We used deletions to map *mus312* genetically to polytene chromosome bands 65E10-F2, a small region on chromosome 3 containing about 50 predicted genes (see Experimental Procedures). We fortuitously identified one of these genes, *CG8601*, in a yeast two-hybrid screen using full-length MEI-9 as bait. Among 26 positive clones, four contained inserts from *Drosophila Ercc1*. This result confirms a previously reported interaction between *Drosophila* MEI-9 and ERCC1 (Sekelsky et al., 2000) and demonstrates the ability of the MEI-9 fusion protein used in the yeast two-hybrid approach to interact with biologically relevant partners. Only one of the remaining 22 clones recovered in the screen generated an interaction as strong as that of the *Ercc1*-containing clones. Sequencing of this clone showed that it contains a segment of the predicted gene *CG8601*, which maps to polytene chromosome band 65F2. To determine whether *CG8601* is in fact *mus312*, we sequenced the protein-coding region of *CG8601* from our three *mus312* mutants. Each of the three *mus312* alleles harbors a nonsense mutation in *CG8601* (Figure 2A), so we conclude that *CG8601* is indeed *mus312*.

The clone recovered in the two-hybrid screen predicts a fusion protein containing residues 61–303 of MUS312. To validate the two-hybrid interaction between MEI-9 and MUS312, we conducted a pull-down assay. We made a construct that expresses MUS312 residues 1–387 fused to *E. coli* glutathione-S-transferase (GST–MUS312). When this fusion protein is immobilized on glutathione agarose beads, it binds FLAG-tagged MEI-9 from nuclear extracts (Figure 2B), indicating a robust and specific interaction between MEI-9 and the amino-terminal region of MUS312.

The *Drosophila* EST collection contains nine *mus312* cDNAs, from a variety of tissues and developmental stages: three from embryos, two from larvae and pupae, two from adult heads, and three from adult testis. We sequenced a full-length embryonic cDNA and determined that it comprises six exons that encode a polypeptide of 1149 amino acid residues. The only identifiable motifs in the predicted MUS312 amino acid sequence are four putative nuclear localization signals, at residues 76–79 (KPKK), 399–402 (KRPK), 737–747 (PVTKKR), and 952–956 (PLKRK). Similarity searches fail to identify homologs of MUS312 in any other organism.

There are several possible explanations for the failure to identify homologs by sequence similarity searches. First, MUS312 may represent a function that is specific to dipteran insects. This seems unlikely given the high

conservation of DNA recombination and repair pathways across eukaryotes. Second, it is possible that sequences of MUS312 homologs do not yet appear in the sequence databases. This also seems unlikely, given the number of complete or nearly complete genome sequences currently available. Finally, it is possible that MUS312 homologs have diverged in amino acid sequence so that they cannot be detected by similarity searches. For example, the *S. pombe* Holliday junction cutting enzyme Mus81 requires the presence of a second subunit, Eme1 (Boddy et al., 2001). Although the *S. cerevisiae* homolog of Eme1, Mms4p, has been identified experimentally (Kaliraman et al., 2001), sequence similarity between these homologs is not detected in standard searches. Identification of MUS312 homologs is therefore likely to require functional or biochemical approaches rather than sequence-based approaches.

The MEI-9–MUS312 Interaction Is Essential for Meiotic Recombination

The MEI-9–MUS312 interaction that we observed in the assays described above presumably reveals an important functional interaction. We determined that the in vivo interaction between MEI-9 and MUS312 is important for their function by characterizing a *mei-9* mutation that disrupts this interaction. The *mei-9*^{12} mutation was recovered in a random chemical mutagenesis screen for meiotic mutations on the X chromosome (K. McKim, personal communication). We measured an X chromosome nondisjunction rate of 23% (n = 1100) for *mei-9*^{12} mutants, a rate almost identical to that of *mus312* mutants (see Table 1).

In contrast to its severe effect on meiotic function, *mei-9*^{12} showed little or no sensitivity to MMS, HN2, or UV (Figure 1). Analysis of several other *mei-9* alleles indicates that partial loss-of-function mutations have much more severe effects in the mutagen survival assays than in the meiotic chromosome nondisjunction assay (Ö.Y. and J.J.S., unpublished data). It is therefore unlikely that the *mei-9*^{12} phenotype represents a general decrease in overall gene function. Rather, *mei-9*^{12} is a true separation-of-function mutation that severely or completely destroys the meiotic function of MEI-9, while having no detectable effect on DNA repair functions.

We sequenced *mei-9*^{12} and found a single base-pair change that predicts a substitution of glutamic acid for glycine at position 306 in the MEI-9 protein (Figure 2C). We considered the possibility that the meiosis-specific phenotype may be caused by inability of MEI-9^{12} to interact with MUS312 during resolution of meiotic recombination intermediates into crossovers. We tested this hypothesis in the yeast two-hybrid assay (Figure 2D). MEI-9^{12} fully interacts with the NER partner ERCC1 but shows no detectable interaction with MUS312. A single amino acid substitution is therefore sufficient to disrupt the interaction between MEI-9 and MUS312, which in turn destroys the function of this complex in meiotic crossover formation.

Experiments with HN2 showed that MEI-9 is required for a subset of MUS312-dependent ICL repair (Figure 2B). Since the primary defect in *mei-9*^{12} flies is apparently inability of MEI-9 to interact with MUS312, the lack of hypersensitivity of *mei-9*^{12} mutants to HN2 suggests that MEI-9 and MUS312 do not need to interact physically in this repair pathway. Alternatively, it is possible that an interaction is necessary, but can be mediated through other proteins in a repair complex. It will be important to determine what other repair proteins can interact with MUS312, and whether the different sensitivities of *mei-9* and *mus312* to HN2 represent completely separable functions in the repair of interstrand crosslinks.

In summary, we have shown that MUS312, like MEI-9, is required late in the meiotic recombination pathway to generate crossovers. A physical interaction between MEI-9 and MUS312 is essential for this function. We have also shown that both MUS312 and MEI-9 are involved in the repair of ICLs, and MUS312 has an additional role in ICL repair independent of MEI-9. We previously proposed that MEI-9 acts on Holliday junctions during meiotic recombination (Sekelsky et al., 1995). The *S. cerevisiae* homolog, Rad1p, is not required to generate meiotic crossovers (Snow, 1968). Although Rad1p has been reported to cleave Holliday junctions in vitro in the absence of its partner Rad10p (Habraken et al., 1994), others have been unable to reproduce this activity (Davies et al., 1995). It is possible that it is the interaction with MUS312 that allows MEI-9 to cut Holliday junctions. MUS312 could affect the activity of the MEI-9–ERCC1 heterodimer, or MUS312 could replace ERCC1, resulting in a MEI-9–MUS312 heterodimer that has Holliday junction cleavage activity. It will be important to distinguish between these possibilities to gain insight into substrate recognition and cleavage by this important family of DNA repair enzymes.

Experimental Procedures

Drosophila Stocks and Genetics

Flies were reared on standard medium at 25°C. Genetic loci not described in the text are described in Flybase (Flybase, 2001). Two alleles of *mei-9* were used: *mei-9*^{A2} is a nonsense mutation that does not produce detectable MEI-9 protein (Ö.Y. and J.J.S., unpublished data); *mei-9*^{12} is a novel missense mutation (see text). X chromosome nondisjunction values were determined as in Sekelsky et al. (1999). Crossing over on chromosome 2 was measured as described previously (Sekelsky et al., 1995).

The two new alleles of *mus312*, *mus312*^{Z1973} and *mus312*^{Z3997}, were recovered from a collection of EMS-treated third chromosome stocks maintained by Ed Koundakjian and Charles Zuker. The collection was screened for HN2 and MMS sensitivity, leading to the identification of these two alleles (A. Laurençon, R.S. Hawley, and K. Burtis, personal communication).

Sensitivity of developing larvae to DNA damaging agents was performed as in Boyd et al. (1976a). For nitrogen mustard and MMS, adults were crossed in plastic shell vials such that mutant and control class were generated among the progeny. Adults were removed after 2 days of egg laying. After 1 additional day, 250 μl of 0.001%–0.008% of the nitrogen mustard mechloramine (HN2, Sigma) in water, or 250 μl of 0.025%–0.05% MMS (Sigma) in water, was added to the medium. For UV, embryos were collected on grape agar plates overnight, then allowed to develop for 4 days. The resulting 2nd and 3rd instar larvae were washed and spread in a monolayer on chilled petri plates, then irradiated in a Stratalinker (Stratagene). To calculate sensitivity, adult progeny were counted, and the ratio of mutant progeny to control was determined. Controls were balanced, heterozygous siblings in the same vial; all *mei-9* and *mus312* alleles used herein are completely recessive for these phenotypes (Boyd et al., 1981; Green, 1981). For the double mutant experiment in Figure 1D, *mei-9*^{A2}*/FM7; mus312*^{Z1973}*/TM3* females were crossed to *mei-9*^{A2}*/Y; mus312*^{Z1973}*/TM3* males. Female progeny with the genotype *mei-9*^{A2}*/FM7; mus312*^{Z1973}*/TM3* (control), *mei-9*^{A2}*/*

mei-9^{A2}; mus312^{Z1973}/TM3 (*mei-9* single mutant), *mei-9^{A2}/FM7; mus312^{Z1973}/mus312^{Z1973}* (*mus312* single mutant), and *mei-9^{A2}/mei-9^{A2}; mus312^{Z1973}/mus312^{Z1973}* (double mutant) were counted. Heterozygosity for *mei-9* did not affect survival of *mus312* mutants and vice versa (data not shown). At least 500 individuals were counted for each point. Numbers are expressed as a percent of expected, based on ratios of progeny classes from untreated controls. Bars represent standard deviation for at least two different experiments, done on different days, with each experiment involving at least eight separate vials for each mutagen concentration.

Yeast Two-Hybrid Screen

We used the two-hybrid vectors and strains described in James et al. (1996). The complete *mei-9* protein-coding region was cloned into pGBD-C1 to express a fusion protein consisting of Gal4p (1-225) and MEI-9. Approximately 1.2×10^6 transformants of a hybrid library in pGAD10, containing 0–4 hr *Drosophila* embryo cDNAs (Dahanukar et al., 1999) were screened (S.M. and J.J.S., unpublished data). Colonies that grew on plates lacking histidine were streaked onto plates lacking adenine. Those that grew were put through a series of additional tests, including isolating and re-transforming the activation domain plasmid, and testing the specificity of the putative interaction. Among 26 clones that passed all tests, five grew more quickly than the others, suggesting a stronger interaction. Four of these clones contained *Ercc1*, and the fifth contained *CG8601/mus312*.

Sequencing of Mutants

The *CG8601* protein coding region was sequenced for the mutations *mus312^{D1}*, *mus312^{Z1973}*, and *mus312^{Z3997}*. Individual flies homozygous for the mutation were homogenized and PCR was performed using gene-specific primers. PCR products were isolated on an agarose gel, purified, and sequenced directly. Mutations were confirmed by sequencing the opposite strand from an independent amplification. For *mus312^{Z1973}* and *mus312^{Z3997}*, the sequence of the progenitor chromosome was also determined. A similar strategy was followed for *mei-9^{12}*, and the sequence of the progenitor chromosome for this mutation was also determined.

GST Pull-Down Experiments

The 5′ end of *mus312* was cloned into the vector pGEX-KG (Frangioni and Neel, 1993) to express a protein in which the first 387 amino acid residues were fused to the carboxy terminus of glutathione S-transferase (GST). GST-MUS312$^{1-387}$ and GST alone were expressed in *E. coli* and purified by binding to glutathione-sepharose beads (Sigma). Nuclear extract from *Drosophila* S2 cells overexpressing FLAG-tagged MEI-9 (S.M. and J.J.S., unpublished data) were incubated with the bound beads. The beads were then washed thoroughly at high salt (300–500 mM NaCl). Bound proteins were eluted by boiling in SDS sample buffer, separated by electrophoresis, transferred to PVDF membrane, and detected with specific antibodies (monoclonal anti-FLAG and polyclonal anti-MEI-9 gave similar results).

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