

# Unique invasions and resolutions: DNA repair proteins in meiotic recombination in *Drosophila melanogaster*

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**Abstract.** To ensure the accurate disjunction of homologous chromosomes during meiosis, most eukaryotes rely on physical connections called chiasmata, which form at sites of crossing over. In the absence of crossing over, homologs may segregate randomly, resulting in high frequencies of aneuploid gametes. The process of meiotic recombination poses unique problems for the cell that must be overcome to ensure normal disjunction of homologous chromosomes. How is it ensured that crossovers occur between homologous chromosomes, rather than between sister chromatids? What determines the number and location

of crossovers? The functions of DNA repair proteins hold some of the answers to these questions. In this review, we discuss DNA repair proteins that function in meiotic recombination in *Drosophila melanogaster*. We emphasize the processes of strand invasion and Holliday junction resolution in order to shed light on the questions raised above. Also, we compare the variety of ways several eukaryotes perform these processes and the different proteins they require.

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## Meiotic recombination in *Drosophila*

*Drosophila melanogaster* has been used as a model organism to study recombination and meiosis for nearly a century. T.H. Morgan first proposed that linkage relationships are a consequence of genes being on the same chromosome and he suggested that Janssens' chiasmotype theory could explain how linked genes can segregate away from one another (Morgan, 1911). Morgan's student Calvin Bridges analyzed cases in which meiotic chromosomes failed to segregate properly to prove the chromosome theory of heredity (Bridges, 1916). Another student of Morgan, Alfred Sturtevant, used recombination rates between different genes to produce the world's first genetic map (Sturtevant, 1913). Sturtevant, Bridges, and Morgan later

used their maps to argue that genes are arranged in a linear order along a chromosome (Sturtevant et al., 1919).

A major advantage of studying meiotic recombination in *Drosophila* is that there are only four chromosomes in the haploid set and aneuploidy is tolerated for the sex chromosomes and for the small chromosome 4. This makes it possible to recover the products of aberrant meiosis in viable offspring. Indeed, most meiotic mutations were recovered on the basis of elevated levels of nondisjunction of the female X chromosomes, which is detected in the adult progeny of those females (Sandler et al., 1968; Baker and Carpenter, 1972). Larry Sandler and his students identified a number of meiotic recombination mutations in screens for elevated levels of X chromosome nondisjunction (Sandler et al., 1968; Baker and Carpenter, 1972). Subsequent studies revealed that some of these also caused hypersensitivity to DNA damaging agents or increased mitotic recombination (Baker et al., 1978). This was one of the first demonstrations of the overlap between DNA repair genes and meiotic recombination genes.

Current models for meiotic recombination are based on the double-strand break repair (DSBR) model of Szostak and colleagues (Szostak et al., 1983). According to this model, meiotic

Received 11 November 2003; manuscript accepted 6 February 2004.

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recombination is initiated by a DSB on one chromatid (Fig. 1). In the budding yeast *Saccharomyces cerevisiae*, meiotic double-strand breaks (DSBs) have been observed at recombination hotspots, and Spo11 has been identified as the enzyme that makes the DSBs (Keeney et al., 1997). The absence of meiotic recombination hotspots in *Drosophila* has precluded physical studies of recombination in this organism. However, mutations in *mei-W68*, which encodes the *Drosophila* homolog of Spo11, abolish meiotic recombination (McKim and Hayashi-Hagihara, 1998; McKim et al., 1998), which suggests that recombination in *Drosophila* is also most likely initiated by DSB formation.

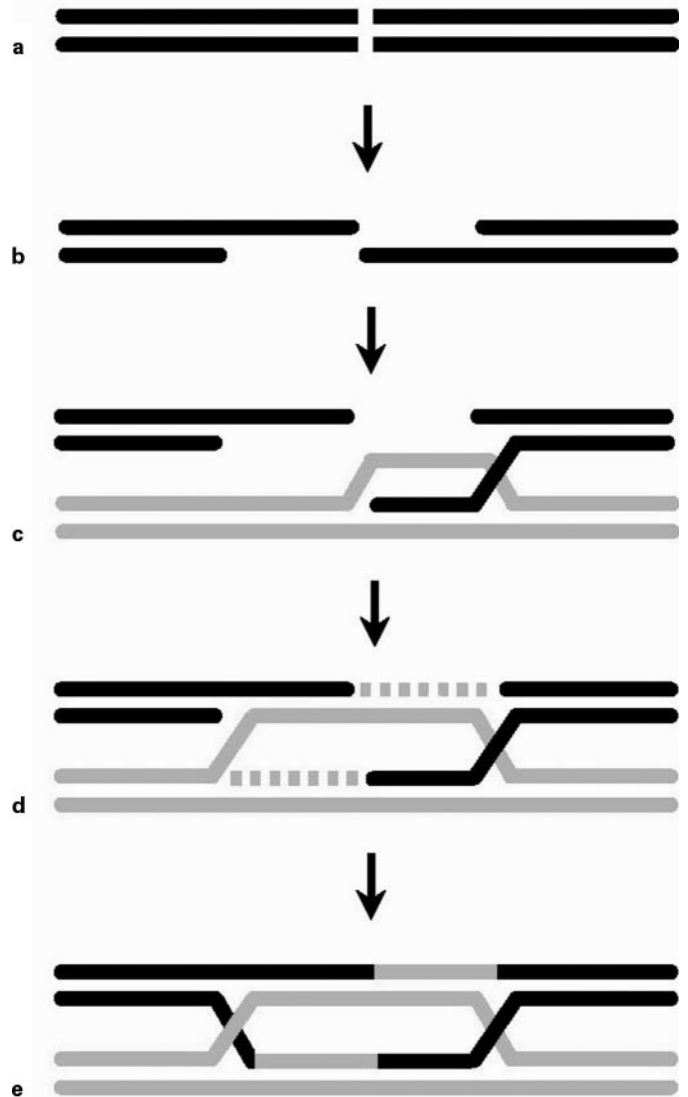
After formation of a DSB, the 5' ends are resected to leave long 3'-ended single-stranded overhangs. One of these overhanging ends invades a homologous, non-sister duplex and primes repair DNA synthesis. The strand displaced by the migrating synthesis bubble can be captured by the other 3' overhang, which primes synthesis using this displaced strand as a template. Ligation of the newly synthesized ends to the resected 5' ends produces an intermediate with two Holliday junctions (HJs) and adjacent heteroduplex DNA (Fig. 1), the central intermediate on the pathway to generating meiotic crossovers. We will first consider strand invasion, one of the key steps in generating this intermediate, and will then turn to a discussion of resolution of the double-HJ intermediate to generate crossovers.

### The strand invasion proteins Rad51 and Dmc1

The business of homologous recombination truly begins when the single-stranded DNA generated by resection of DSB ends invades a homologous duplex DNA molecule to prime repair synthesis. In *S. cerevisiae*, the key protein that catalyzes strand invasion is Rad51 (reviewed in Sung et al., 2003). Rad51 has sequence similarity to the *E. coli* recombination protein RecA (Shinohara et al., 1992), and is highly conserved throughout eukaryotes (Fig. 2). Like RecA, Rad51 coats single-stranded DNA to make a filament, which invades a homologous duplex DNA, displacing one strand and base-pairing with the other. Repair of mitotic DSBs by homologous recombination requires Rad51, and *rad51* mutations result in severe hypersensitivity to ionizing radiation (reviewed in Symington, 2002).

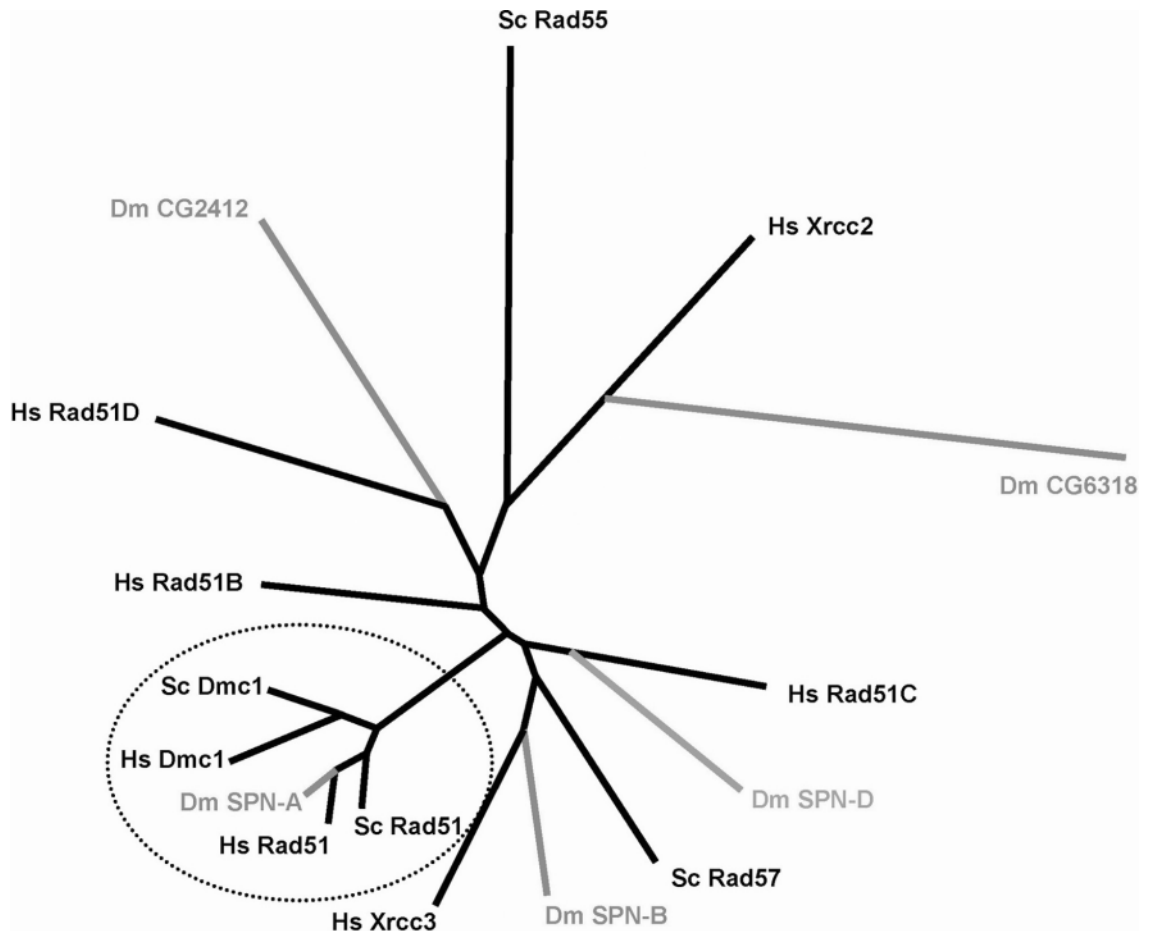
In addition to Rad51 meiotic recombination in *S. cerevisiae* requires a second strand invasion protein, Dmc1. Dmc1 is highly homologous to Rad51 but is expressed exclusively during meiosis (Bishop et al., 1992). Like Rad51, Dmc1 can catalyze strand invasion in vitro though with less activity than Rad51 (Li et al., 1997). One important function of Dmc1 seems to be to ensure that recombination events occur between homologous chromosomes rather than between sister chromatids (Schwacha and Kleckner, 1997).

Although meiosis-specific Dmc1 orthologs are present in many species including mice, humans, *Arabidopsis thaliana*, and *S. pombe* (Klimyuk and Jones, 1997; Yoshida et al., 1998; Fukushima et al., 2000), the genome of *Drosophila melanogaster* conspicuously lacks a Dmc1 ortholog (Sekelsky et al., 2000).



**Fig. 1.** Double Holliday junction formation based on the DSB model for meiotic recombination. (a) Meiotic recombination begins with a double-strand break on one chromatid. (b) The 5' ends are resected to leave 3' single-stranded tails. (c) One of these 3' ends invades the homologous chromosome to prime repair DNA synthesis. (d) The other end of the break can capture the displaced D-loop and also prime repair synthesis (stippled line represents new synthesis). (e) Ligation of the ends results in an intermediate with two Holliday junctions and heteroduplex DNA.

If meiotic recombination occurs preferentially between homologous chromosomes in *Drosophila*, as classical genetic studies indicate, how might this be ensured in the absence of Dmc1? One possibility is that other Rad51 family members confer this bias. There are two Rad51 paralogs, *spn-D* and *CG6318*, that are expressed exclusively in the female germline (Staeva-Vieira et al., 2003). Based on sequence comparisons (see below), we think it unlikely that these proteins have acquired strand invasion functions similar to that of Dmc1. However, there have been no biochemical studies of SPN-D or CG6318 to address this issue. It is also possible that DmRad51, which is encoded by the *spn-A* gene (Staeva-Vieira et al., 2003), retains some of



**Fig. 2.** A cladogram of the RecA related proteins in *S. cerevisiae*, *Drosophila*, and humans. *Drosophila* homologs are shown in grey. The dotted circle highlights proteins that have been shown to have strand exchange activity. Note that *Drosophila* lacks a DMC1 ortholog.

the Dmc1 properties, as has been suggested based on sequence comparisons (Villeneuve and Hillers, 2001).

In our view, a more likely alternative concerns a fundamental difference between the progression of meiotic recombination in *Drosophila* versus that of other organisms. In *S. cerevisiae*, initiation of recombination occurs prior to chromosome synapsis and Dmc1 (along with other strand-exchange proteins) is required to achieve synapsis (reviewed in Hunter, 2003). In *Drosophila*, however, chromosomes achieve full synapsis before recombination is initiated (McKim et al., 1998; Jang et al., 2003). This is also the case in *C. elegans* which also lacks a Dmc1 ortholog (Dernburg et al., 1998; Colaiacovo et al., 2003). In *Drosophila* and *C. elegans*, the structure of the synapsed homologs could ensure that recombination events occur between homologs rather than between sister chromatids. However, Colaiacovo et al. (2003) recently reported that a complete synaptonemal complex is not required to ensure the preference for homologous chromosome recombination in *C. elegans*.

### Rad51 paralogs

In addition to Rad51 and Dmc1, the *S. cerevisiae* genome encodes two Rad51-related proteins: Rad55 and Rad57. Rad55 and Rad57 alone do not have strand invasion activity; rather, they appear to function as a heterodimer that contributes to assembly or stability of the Rad51 filament (reviewed in Sung et al., 2003). Like *rad51* mutations, *rad55* and *rad57* mutations confer hypersensitivity to ionizing radiation and an inability to complete meiotic recombination. Vertebrate genomes encode five such Rad51 paralogs (Xrcc2, Xrcc3, Rad51B, Rad51C, and Rad51D), and the *Drosophila* genome has four (SPN-B, SPN-D, CG2412, and CG6318). Comparisons of the sequences of the conserved regions of Rad51 family members generates a tree with three major branches (Fig. 2). The first is occupied by the true strand exchange proteins, Rad51 and Dmc1. The other two branches each have one *S. cerevisiae* paralog, two human paralogs, and two *Drosophila* paralogs (human Rad51B may lie between these branches, or may be found on the Rad55 branch).

Until five years ago, there were no known mutations in *Drosophila* Rad51 family members. The first meiotic phenotype to

be reported for this family came from an unexpected source: studies of oocyte pattern formation. To understand how studies of oocyte patterning led to the identity of Rad51 paralogs in *Drosophila*, it is important to be familiar with the developmental context in which meiosis occurs. In *Drosophila*, meiotic recombination occurs only in females. In male meiosis homologous chromosomes are segregated through a poorly understood achiasmate mechanism. The *Drosophila* ovary consists of two clusters of ovarioles, each of which contains a series of progressively more developed egg chambers. Oogenesis initiates at the anterior end of each ovariole when a germline stem cell divides to give rise to a cystoblast. Prior to meiosis the cystoblast undergoes four mitotic divisions with incomplete cytokinesis resulting in a 16-cell cyst in which the cytoplasm of adjacent cells is connected by ring canals. The two cells with four ring canals are designated as pro-oocytes and enter meiotic prophase. The chromosomes of the pro-oocytes condense and synapse with complete synaptonemal complex formation, and meiotic recombination is initiated during this pachytene stage. At some point during pachytene, one of the pro-oocytes exits meiosis and joins the other 14 cells in the cyst in following the nurse cell fate, leaving a single oocyte per cyst. As each cyst moves toward the posterior it becomes encapsulated by somatic follicle cells.

The follicle cells that encapsulate the germline cyst will secrete the eggshell around the oocyte. Patterning of the eggshell depends on two signaling events between the oocyte nucleus and the follicle cells. This patterning requires two separate signaling events both of which require localization of Gurken (GRK, an epidermal growth factor receptor ligand) around the oocyte nucleus. The first event finds the oocyte nucleus positioned at the posterior of the oocyte allowing GRK to signal the surrounding follicle cells to assume a posterior fate. The oocyte nucleus, surrounded by GRK, then migrates to an anterior corner of the oocyte where GRK signals nearby follicle cells to assume dorsal characteristics (Riechmann and Ephrussi, 2001).

It is this process – patterning of the eggshell through communication between the oocyte nucleus and the surrounding follicle cells – that led to the discovery of phenotypes associated with Rad51 paralogs in *Drosophila*. A number of mutations that affect both the anterior-posterior and dorsal-ventral patterning of the eggshell have been identified. Because of the characteristic shape of the eggshell, mutations in this class that mapped to chromosome 2 were named after vegetables with similar shapes (*okra*, *aubergine*, *gurken*, etc.), whereas those on chromosome 3 were named for their resemblance to the spindle of a spinning wheel (*spn-A* through *spn-F*). To identify components of the GRK signaling pathway, Ghabrial and coworkers (Ghabrial et al., 1998) sought to clone *okra* (*okr*) and *spn-B*. To their surprise, they found that *spn-B* encodes a Rad51 paralog most closely related to mammalian Xrcc3, and *okra* encodes the homolog of Rad54, which is also involved in facilitating strand invasion in vivo. More recently, *spn-D*, was shown to encode a Rad51 paralog most closely related to human Rad51C (Abdu et al., 2003), and *spn-A* was found to encode the Rad51 ortholog (Staeva-Vieira et al., 2003).

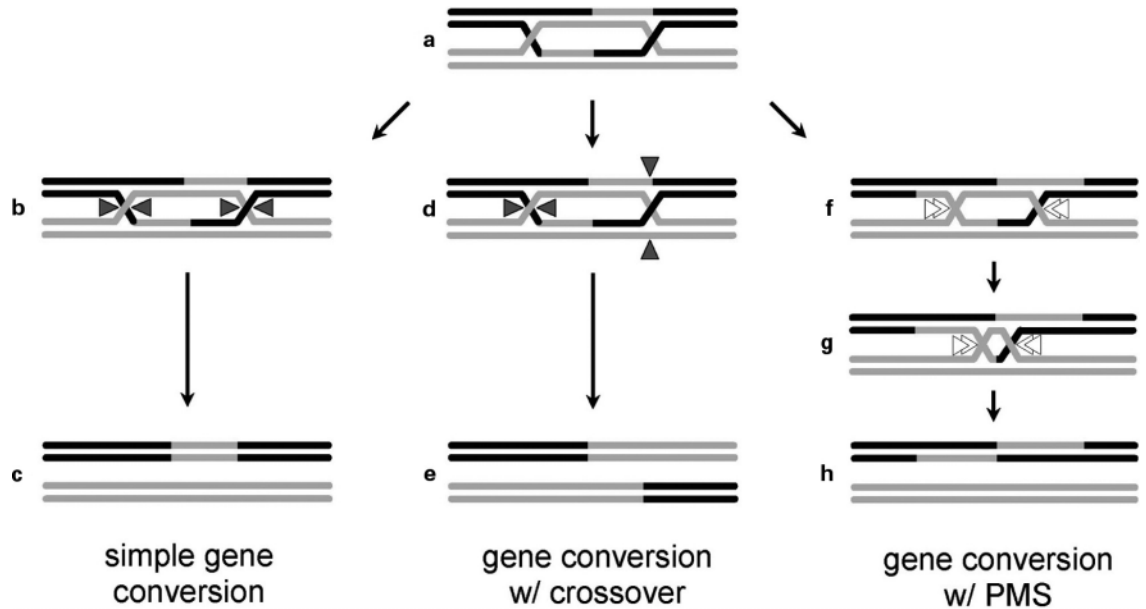
Why do mutations in strand invasion genes lead to eggshell patterning defects? In *S. cerevisiae* *rad51* mutations result in meiotic cell cycle arrest after resection of DSBs in what is termed the pachytene checkpoint (reviewed in Roeder and Bailis, 2000). Ghabrial and colleagues proposed that a similar checkpoint in *Drosophila* could disrupt the subsequent oocyte-follicle cell signaling (Ghabrial and Schupbach, 1999). To test this hypothesis, they first showed that mutations in *mei-W68* are epistatic to *okr* and *spn-B* mutations: Double mutants have the *mei-W68* phenotype, indicating that patterning defects are only seen if DSBs have been made. They then demonstrated that mutations in *mei-41*, which encodes the *Drosophila* ortholog of the checkpoint kinase Atr (Hari et al., 1995) suppress the patterning defects of *okr* and *spn-B* mutants. They concluded that in the absence of DmRad54 or SPN-B, DSBs remain unrepaired and activation of a DNA damage checkpoint alters cell cycle progression which disrupts signaling between the oocyte nucleus and the somatic follicle cells.

Further studies of Rad51 paralogs in *Drosophila* are likely to result in more interesting insights into the functions of these proteins in DNA repair and meiotic recombination. Two of the four paralog genes, *spn-D* and *CG6318*, are expressed only in the female germline, and thus might have meiosis-specific functions. The other two paralogs, *spn-B* and *CG2412*, are more widely expressed. However, *spn-B* mutants do not exhibit strong defects in DNA repair, in spite of the strong meiotic phenotype of these mutants (Ghabrial et al., 1998). *CG2412* is uncharacterized, but may correspond to the DNA repair gene *rad201* (JS, unpublished data). Mutations in this gene cause hypersensitivity to DNA damaging agents but do not cause meiotic defects. Genetic and physical studies of the *Drosophila* Rad51 paralogs should help to shed light on their different functions.

### Resolution of double Holliday junction intermediates in *Drosophila*

A key intermediate in the generation of meiotic crossovers is the double-Holliday junction (DHJ), which has two Holliday junctions and associated heteroduplex DNA (Fig. 3). According to the canonical DSBR model, resolution of the DHJ occurs by symmetrical nicking of the DNA at each junction. Cutting of the same two strands at both junctions results in non-crossover products, whereas cutting of different strands at each junction (i.e., each of the four strands is cut once) results in crossover products.

Resolution of the DHJ intermediate is perhaps the most poorly understood step in recombination. This is in part due to the failure to identify candidates for Holliday junction resolvases – enzymes that cut HJs (reviewed in Heyer et al., 2003). One candidate for an HJ resolvase that was identified recently is the *S. pombe* Mus81–Eme1 endonuclease. This enzyme can cut HJs in vitro, and mutations in *mus81* or *eme1* cause a meiotic phenotype similar to what one would expect for a defect in resolution of recombination intermediates (Boddy et al., 2001). However, purified Mus81–Eme1 cuts nicked and gapped HJs more efficiently than intact HJs (Osman et al.,



**Fig. 3.** Three models for generating recombination products from a double Holliday junction intermediate. **a–e** correspond to the DSBR model of recombination. To generate a gene conversion two strands of DNA are nicked twice, once at each Holliday junction (**b**), heteroduplex is repaired and the ends are ligated (**c**). To create a gene conversion with associated crossover of flanking DNA, each strand is nicked once (**d**), heteroduplex DNA is repaired and the ends are ligated (**e**). (**f–h**) Model for double-Holliday junction dissolution in the absence of MEI-9. Holliday junctions are branch migrated toward each other (**f, g**). Remaining catenations are removed by a topoisomerase activity, leaving heteroduplex DNA on only one duplex flanking the site of the original break (**h**).

2003). This has led Osman and colleagues to propose that Mus81–Eme1 generates crossovers not by cutting HJs, but by nicking a single-end invasion intermediate that occurs prior to double Holliday junction formation (reviewed in Hollingsworth and Brill, 2004).

Based on genetic studies, the *Drosophila* MEI-9 protein is a strong candidate for a Holliday junction resolvase (Sekelsky et al., 1998). MEI-9 is the *Drosophila* ortholog of the nucleotide excision repair (NER) endonuclease XPF (human) and Rad1 (*S. cerevisiae*) (Sekelsky et al., 1995; Sijbers et al., 1996). The nuclease domain conserved in MEI-9, XPF, and Rad1 is structurally similar to that of Mus81 (Sgouros et al., 1999) and like Mus81, XPF and Rad1, together with their respective non-catalytic subunits Ercc1 and Rad10, have DNA structure-specific endonuclease activity in vitro (Bardwell et al., 1994; Park et al., 1995).

The proposal that MEI-9 cuts Holliday junctions during meiotic recombination is based solely on genetic data. Mutations in *mei-9* result in a reduction in crossing over of greater than 90% (Baker and Carpenter, 1972). There are a number of other *Drosophila* meiotic mutations that cause reductions similar in magnitude, but in most cases, the distribution of crossovers along the chromosome is also affected. In contrast, in *mei-9* mutants the reduction is uniform along the chromosome. Based on this observation Baker and Carpenter (1972) concluded that *mei-9* mutants are defective in the actual process of exchange.

In the canonical model, the DHJ intermediate includes heteroduplex DNA (hDNA), regions in which each strand of a duplex is derived from a different homologous chromosome.

Sequence differences between the homologous chromosomes are reflected as base-base mismatches or insertion/deletion heterologies within hDNA. Most heterologies in hDNA are repaired efficiently. Repair can restore the original 2:2 ratio of alleles at a given site or can result in gene conversion – a change to a 3:1 ratio. Because hDNA is associated with the recombination intermediate, gene conversion can occur with either crossover or non-crossover products. The phenomenon of gene conversion allows the detection of some of the non-crossover products of the meiotic recombination process.

In contrast to the strong reduction in crossovers, Carpenter (1982) found that non-crossover recombination is not reduced in *mei-9* mutants. However, she found that mismatches within hDNA were frequently unrepaired in these events. In *Drosophila*, failure to repair hDNA results in a mosaic progeny, in which some tissues have one maternal allele, and others have the other maternal allele. In essence, the two maternal alleles have segregated from one another after meiosis; hence, this outcome is referred to as post-meiotic segregation (PMS).

The meiotic defect in *mei-9* mutants is therefore two-fold: a strong reduction in crossovers without a reduction in non-crossovers, and a failure to repair heterologies in meiotic heteroduplex. Given the similarity between the nuclease domains of MEI-9 and Mus81, it is tempting to speculate that the function of MEI-9 in generating crossovers is similar to that proposed for Mus81 (Osman et al., 2003). In this scenario, the PMS phenotype of *mei-9* mutants represents a second function in repair of hDNA heterologies. A more parsimonious model is that MEI-9 has a single function, and that the lack of crossovers

and the lack of hDNA repair are both consequences of this defect. We propose that MEI-9 is involved in cutting Holliday junctions to resolve recombination intermediates.

If resolution of a DHJ requires cutting, as the canonical model dictates, and MEI-9 is required to make the cuts, how can *mei-9* mutants generate non-crossover recombinants? We propose that in the absence of MEI-9, the DHJ intermediate is "resolved" by branch migration of the junctions toward one another, followed by decatenation (Fig. 3). Wu and Hickson (2003) recently demonstrated that human Blm and topoisomerase IIIa can carry out this reaction in vitro, in a process they refer to as double-junction dissolution. This process generates only non-crossover products. If a similar process can occur in *Drosophila*, any DHJ intermediate slated to become a crossover would instead become a non-crossover. To account for the PMS phenotype observed in *mei-9* mutants, we further propose that at least some repair of hDNA is dependent on the presence of the nicks introduced during HJ resolution. No nicks are introduced during dissolution, so unrepaired heterologies would remain in the non-crossovers arising by this mechanism in *mei-9* mutants, leading to the PMS phenotype.

This model accounts for both aspects of the *mei-9* mutant phenotype as consequences of a single defect in cutting Holliday junctions. It does not, however, address the question of whether MEI-9 is normally involved in the formation of non-crossover recombinants. Studies of meiotic recombination in *S. cerevisiae* suggest that most non-crossovers arise through synthesis-dependent strand annealing (SDSA), a pathway that does not involve formation of Holliday junction intermediates (Allers and Lichten, 2001; Hunter and Kleckner, 2001). In *Drosophila*, SDSA is thought to account for most mitotic DSB repair (Kurkulos et al., 1994; Nassif et al., 1994; Adams et al., 2003), but there are no data concerning the use of SDSA in meiotic recombination. According to our model for MEI-9 function, non-crossover events generated by an SDSA pathway would be expected to be independent of MEI-9, and therefore would not show PMS in a *mei-9* mutant. Carpenter (1982) was able to detect PMS in only 60% of the non-crossover recombinants recovered from *mei-9* mutants. It is possible that the non-crossovers for which PMS could not be detected by Carpenter arose through SDSA. Alternatively, there may be some repair of heterologies that is not dependent on HJ cutting. It is also possible that the techniques available to Carpenter precluded the identification of all cases of PMS. It will be important to use molecular techniques to determine what fraction of non-crossovers have PMS in *mei-9* mutants, and whether non-crossover events with PMS have any regions of full conversion.

At present, there are no biochemical studies to support the hypothesis that MEI-9 cuts Holliday junctions. Indeed, neither XPF-Ercc1 nor Rad1-Rad10 can cut HJs in vitro (A. Sancar, personal communication; Davies et al., 1995). It has been reported that Rad1 cuts Holliday junctions in the absence of Rad10 (Habraken et al., 1994), but other researchers have been unable to repeat this result (West, 1995). It is possible that MEI-9 has acquired a Holliday junction cleavage activity not present in the yeast or mammalian orthologs. Alternatively, MEI-9 may require one or more accessory proteins either in addition to or in place of its normal partner, ERCC1. MUS312

is a protein that interacts physically with MEI-9, and this interaction is required for the generation of meiotic crossovers (Yildiz et al., 2002). Mutations in *mus312* confer a meiotic phenotype similar to that of *mei-9* mutants, including a mutation that specifically disrupts the physical interaction with MEI-9.

Unlike MEI-9, MUS312 is not involved in nucleotide excision repair. Although *mei-9* and *mus312* mutants both exhibit hypersensitivity to the crosslinking agent nitrogen mustard, *mus312* mutants are more sensitive than *mei-9* mutants, and a *mei-9* mutation that disrupts the interaction with MUS312 in vitro is not hypersensitive to nitrogen mustard (Yildiz et al., 2002). Thus, MEI-9 and MUS312 may function as part of a complex in meiotic recombination while having separable roles in DNA repair. MUS312 is a novel protein with no recognizable structural or functional motifs. We have been unable to identify homologs of MUS312 in yeast, *Arabidopsis*, or *C. elegans*, but there appears to be a poorly conserved vertebrate homolog that interacts with XPF (J Larocque and JS, unpublished data). It is unknown at present whether vertebrate MUS312 functions in DNA repair pathways.

A question remaining to be answered is whether MUS312 replaces ERCC1, the nucleotide excision repair partner of MEI-9, in meiotic recombination, or whether MUS312 forms a complex with the MEI-9-ERCC1 heterodimer. Genetic characterization of a recently generated knockout in *Drosophila Ercc1* (S Radford and JS, unpublished data) should provide an answer to this question, but in vitro studies of the biochemical properties of these proteins will be essential to addressing the model that MEI-9 cuts Holliday junctions.

### Crossover control

Understanding the final step in meiotic recombination has also been complicated by the different ways model organisms control the formation of crossovers. In most meiotic systems, crossovers between homologous chromosomes are essential to ensure their accurate disjunction. As such, the distribution of crossovers is tightly controlled. In many organisms, each chromosome arm experiences about one to three crossovers per meiosis, regardless of physical length (reviewed in Hawley, 1988). In addition, crossover positioning along each chromosome arm is regulated, with crossovers being most frequent in the middle of each arm. The biased distribution of crossovers along chromosomes is believed to be related to functional aspects of the chiasmata that result from crossing over (reviewed in Koehler et al., 1996). The distribution is impacted by several elements of the recombination pathway, including strand invasion, resolution, and crossover interference. However, the role of each in determining the outcomes of recombination appears to differ among organisms.

Crossover interference, first observed by Sturtevant in 1913 (Sturtevant, 1913), is a phenomenon whereby the occurrence of a crossover prevents other crossovers from forming nearby. Though the mechanism of crossover interference is unknown, many proteins have been found to be required for normal interference. In *S. cerevisiae*, crossover interference requires the synaptonemal complex (SC), an elaborate network of proteins

**Table 1.** Protein requirements for crossover in organisms with and without interference in crossover distribution

Crossover class	<i>S. pombe</i> <sup>a</sup>	<i>S. cerevisiae</i> <sup>b</sup>	<i>C. elegans</i> <sup>c</sup>	<i>D. melanogaster</i> <sup>d</sup>
I. Msh4/Msh5-dependent	0 %	50–70 %	100 %	0 %
II. Mus81-dependent	100 %	30–50 %	0 %	0 %
III. MEI-9-dependent	0 %	0 %	0 %	90 %

<sup>a</sup> Boddy et al. (2001).

<sup>b</sup> Ross-Macdonald and Roeder (1994), Hollingsworth et al. (1995). The double *msh5 mms4* double mutant has some residual crossovers, but the genetic requirements for these crossovers are unknown.

<sup>c</sup> Zalevsky et al. (1999).

<sup>d</sup> Baker and Carpenter (1972). Genetic requirements for the residual crossovers in *mei-9* mutants are unknown.

that links homologs during pachytene (Sym and Roeder, 1994). In contrast, the fission yeast *S. pombe* does not make complete SC, and does not exhibit crossover interference (Kohli and Bahler, 1994). The relationship between SC and recombination is complex, but provides insight into the different mechanisms used to promote inter-homolog recombination during meiosis. In *S. cerevisiae*, where DSBs occur non-randomly at hotspots, Spo11, Rad51, and Dmc1 are all required for synapsis, implying that DSB formation and strand invasion are essential for SC formation (reviewed in Hunter, 2003). In *C. elegans* and *Drosophila*, organisms in which meiotic recombination hotspots have not been observed, and which lack Dmc1, Spo11 homologs are not required for synapsis (Dernburg et al., 1998; McKim et al., 1998; McKim and Hayashi-Hagihara, 1998; Sekelsky et al., 2000).

Crossover distribution is obviously different in those organisms that show interference and those that do not. This is reflected in the different protein requirements for each type of crossover (Table 1). In *S. pombe*, which lack interference, all crossovers require Mus81 and Eme1 (Boddy et al., 2001). In *S. cerevisiae*, a subset of crossovers requires Mus81 and the Eme1 homolog Mms4 (de los Santos et al., 2003). Crossovers that are dependent on Mus81, like all crossovers in *S. pombe*, do not exhibit interference. Rather, interference in *S. cerevisiae* is restricted to another set of crossovers, those that require the meiosis-specific proteins Msh4 and Msh5 (Ross-Macdonald and Roeder, 1994; Hollingsworth et al., 1995; Novak et al., 2001). These proteins are structurally similar to the *E. coli* mismatch repair protein MutS, but the function of the Msh4/Msh5 heterodimer in promoting crossovers is not well understood. In *C. elegans*, all crossovers require Msh4 and Msh5 (Zalevsky et al., 1999; Kelly et al., 2000).

Among these model organisms, then, there are two types of crossovers – those that exhibit interference and require Msh4–Msh5 (class I) and those that lack interference and require Mus81–Eme1 (Class II). *C. elegans* has only class I crossovers, *S. pombe* has only class II crossovers and *S. cerevisiae* has both (and possibly an uncharacterized third class). In *Drosophila*, Mus81 and Mms4 are not required for any meiotic crossovers (K Trowbridge and JS, unpublished data), and the *Drosophila* genome lacks Msh4 and Msh5 orthologs (Sekelsky et al., 2000). Therefore *Drosophila* has neither class I nor class II crossovers. Instead, about 90% of the crossovers in *Drosophila* require MEI-9 and MUS312. In the absence of MEI-9, the remaining 10% of crossovers still exhibit interference (Baker and Carpenter, 1972); this could represent yet another class of crossovers.

### Concluding remarks

DNA repair proteins in *Drosophila* play significant and unique roles in meiosis. The absence of a Dmc1 ortholog to ensure recombination between homologs and the apparent novel function of the XPF ortholog MEI-9, when interacting with MUS312, make *Drosophila* meiotic recombination distinct from that of other model invertebrates. As more genetic and biochemical data on meiotic recombination in different organisms emerge, it is becoming clear that the enzymatic activities required for recombination are conserved, even though the specific proteins that perform these activities often differ. Many questions about meiosis and meiotic recombination remain. It is important to continue the search for answers in a variety of different organisms, including *Drosophila melanogaster*.

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