Damage Control: The Pleiotropy of DNA Repair Genes in *Drosophila melanogaster*

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THE responses to DNA damage in eukaryotes are complex, involving multiple overlapping and intersecting pathways. It has become increasingly evident that even the best understood DNA repair pathways have unforeseen levels of complexities, and that some components of these pathways have additional functions in other processes such as replication, transcription, meiotic recombination, and gene silencing. Studies of DNA repair genes and their products in *Drosophila melanogaster*, with its extensive array of genetic tools, have the potential to provide new inroads into understanding the multiple roles of DNA repair enzymes in eukaroytes.

The study of DNA repair in Drosophila began with the convergence of two types of mutant screens. In the first case, Lindsley and Sandler and their collaborators (Sandler *et al.* 1968; Baker and Carpenter 1972) searched for meiotic mutants (*mei*), some of which decreased the frequency of meiotic recombination. In the second effort, Boyd *et al.* (1976a; Smith 1976; Boyd *et al.* 1981; Henderson *et al.* 1987) began to specifically dissect the repair processes by screening for mutagen-sensitive mutants (*mus*) whose phenotype was defined by a greatly heightened susceptibility to various mutagenic agents.

Perhaps the cornerstone of work on Drosophila DNA repair was laid in a classic paper by Baker *et al.* (1976), demonstrating that a large fraction of mutations isolated on the basis of a meiotic recombination defect conferred mutagen hypersensitivity, and vice versa, and by a series of collaborative papers in which Baker and Gatti and others elegantly demonstrated that many of the *mei* or *mus* mutants also exhibited severe defects in mitotic chromosome behavior (Baker *et al.* 1978; Gatti *et al.* 1980). These findings led to the now widely held view that genes defined by repair-defective mutations are less likely to define functions specifically involved in the repair of mutagen-induced damage than they are to define essential or important functions in the normal DNA metabolism of the organism. In this review we discuss several cases in which genetic studies of DNA repair genes in Drosophila have yielded unique insights into their functions in both mutagenized and untreated cells.

mus309 and the recognition of double-strand breaks: The first step in the response to DNA damage is the detection of the damage. Specialized proteins that recognize different classes of DNA damage have been iden-

tified in many organisms. For example, the human XPA protein is believed to mediate recognition of intrastrand crosslinks (reviewed in Wood 1996), and *Escherichia coli* MutS (and the eukaryotic Msh proteins) binds specifically to base pair mismatches and small insertion/deletion mutations (reviewed in Modrich and Lahue 1996). In this section, we discuss the Drosophila homolog of Ku, a protein implicated in the recognition and repair of DNA double-strand breaks (DSBs).

DSBs can be repaired in either of two general ways. If a sequence with homology to the broken end exists, recombinational repair is possible, to yield either simple gene conversion or reciprocal exchange. Alternatively, the ends can be joined together without consulting external homologies. In *Saccharomyces cerevisiae*, recombinational repair dependent on genes in the *RAD52* epistasis group predominates. The existence of an end-joining pathway can also be demonstrated but is only evident in *rad52* mutants (Boulton and Jackson 1996; Milne *et al.* 1996; Barnes and Rio 1997). By contrast, DSB repair in mammals seems to occur primarily through an end-joining reaction. An intriguing intersection between these repair strategies occurs in Drosophila.

In mammalian cells, DSB end-joining requires DNA-dependent protein kinase (DNA-PK), a complex comprising a catalytic subunit and Ku (reviewed in Jeggo *et al.* 1995). Ku was first identified as an antigen in patients with autoimmune disorders (Mimori *et al.* 1981). This heterodimer of 70-kDa and 80-kDa subunits binds in a sequence-independent manner to DNA ends and in a sequence-dependent manner to internal sites (Knuth *et al.* 1990; Messier *et al.* 1993). In addition to its role in DSB repair, DNK-PK functions in *V(D)J* recombination, the site-specific recombination mechanism through which diverse immunoglobulin genes and T cell receptor gene segments are created (reviewed in Jeggo *et al.* 1995).

The Drosophila homolog of Ku70 was identified initially as inverted repeat binding protein (IRBP), a protein that binds to *P*-element inverted repeats (IRs; Rio and Rubin 1988; Beall *et al.* 1994). Coincidentally, much of our knowledge of pathways used to repair DSBs in Drosophila comes from studies using *P* elements. Genetic and molecular studies have indicated that these elements transpose via a conservative, cut-and-paste mechanism, in which transposase excises an element from a donor site, leaving a DSB (Figure 1), and then integrates that element into a target site (Engels *et al.* 1990; Kaufman and Rio 1992). Repair of the DSB
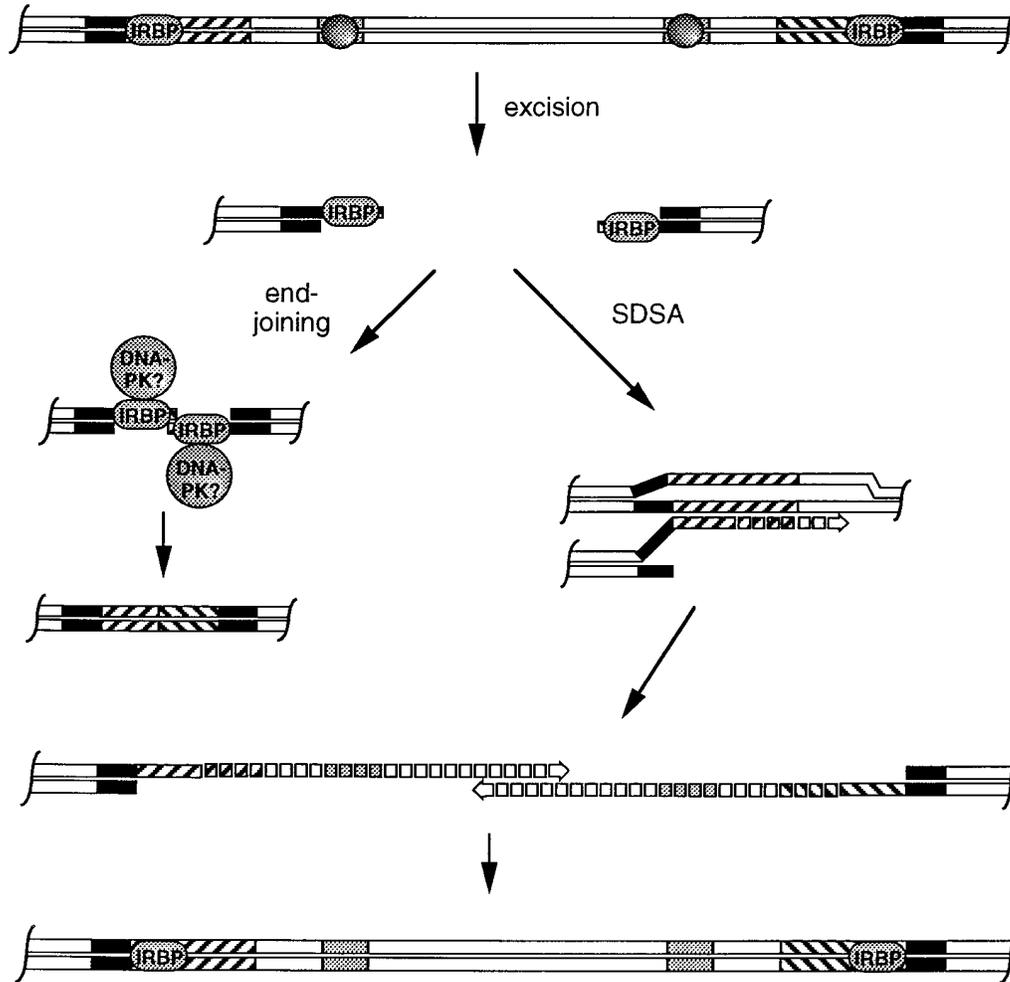
Figure 1.—Some possible roles for IRBP/Ku in the repair of double-strand breaks following *P*-element excision. A schematic of a *P*-element insertion is shown in double-stranded form at the top, with 31-bp inverted repeats hatched and the 8-bp target site duplication in black. IRBP is indicated as an ellipsoid bound to the outer half of each IR; P transposase (small spheres) binds to sites (stippled) internal to the IRs. Excision leaves a 17-nt 3′ single-stranded tail attached to each target site duplication. Two modes of repair are diagrammed: On the left is shown an example of end-joining, perhaps mediated by IRBP and DNA-PK_{cs} (large spheres), resulting in about half of each IR remaining at the donor site (Stavely *et al.* 1995). On the right is shown recombinational repair, by the synthesis-dependent strand annealing (SDSA) model (Nassif *et al.* 1994), in which the 3′ tail is extended by new synthesis (dashed lines), using in this case the sister chromatid as a template (only the left end is shown; the right end is believed to undergo new synthesis independently). Annealing of overlap between the newly synthesized strands is followed by additional repair synthesis and ligation, resulting in a copy of the original insertion element, or internal deletions, if internal homologies exist (Kurkulos *et al.* 1994).

at the donor site is believed to occur most frequently by simple gene conversion, using either the sister chromatid, the homologous chromosome, or an ectopically located homologous sequence as a template (Engels *et al.* 1990; Gloor *et al.* 1991).

Beall and Rio (1996) showed that IRBP/Ku70 is encoded by the *mus309* locus. In their genetic studies, mutations in *mus309* caused reduced viability of males whose *X* chromosome carried a *P* element undergoing excision, suggesting a defect in the ability to repair breaks resulting from excision. In a plasmid injection assay, defects in both the frequency and the fidelity of end-joining were observed in *mus309* mutants. Hence, loss of IRBP/Ku causes defects in at least the end-joining

pathway for DSB repair, and possibly the recombinational repair pathway also.

As noted above, the predominant repair pathway following *P*-element excision is believed to be a recombinational one that results in simple gene conversion. Excision leaves a 3′ single-stranded overhang composed of the terminal 17 nt of the IR remaining at the donor site (Beall and Rio 1997). This is precisely the sequence to which IRBP binds, at least in its double-stranded form (Rio and Rubin 1988). If IRBP remains bound to this single-stranded tail after excision, it may help to protect the end so as to facilitate repair either by recombination or by end-joining. End-joining without processing of the ends would result in 17 bp of each IR remaining at the

donor site (or fewer, if end-joining occurs through short base-paired overlaps, as seems to be the case). Indeed, such events are frequently observed in assays in which they can be detected (Takasu-Ishikawa *et al.* 1992; Stavely *et al.* 1995). Results of similar *in vivo* assays performed in *mus309* mutants have not yet been reported.

Some features of conversion events following DSB repair are interpreted as resulting from exonucleolytic degradation at the donor site prior to template-directed resynthesis (Gloor *et al.* 1991; Johnson-Schlitz and Engels 1993; Nassif and Engels 1993). Some of these studies relied on the use of a template that does not carry *P*-element ends and therefore may have required some degree of DSB end degradation prior to recombinational repair. In experiments in which *P*-element ends were present on the repair template, however, a preference for unextended gaps was observed (Johnson-Schlitz and Engels 1993). It would be interesting to repeat these assays also in *mus309* mutants, to see whether gaps would remain unextended.

If the 17-nt overhangs are protected prior to recombinational repair, a sequence bearing *P*-element IRs (*e.g.*, the sister chromatid) would be preferentially used as a repair template. This would result in replacement of *P*-element sequences back into the donor site, which, when combined with the forward transposition reaction, would tend to increase *P*-element copy number. Hence, protection of the 17-nt tail by IRBP followed by recombinational repair would be predicted to increase the proportion of sister chromatid-templated repair and therefore the *P*-element copy number. In this scenario, *P* elements may have coopted Ku to aid in increasing their copy number.

Binding of IRBP to *P*-element IRs might also indicate a function of IRBP in the transposition reaction. *P*-element-encoded transposase is the only polypeptide required for the *in vitro* forward transposition reaction (excision and strand transfer). It is possible, however, that transposase activity is modulated *in vivo* by, for example, phosphorylation by the Drosophila homolog of DNA-PK. This possibility is discussed by Beall and Rio (1997), who point out some interesting structural similarities between *P*-element ends and the recombination signal sequences involved in *V(D)J* recombination.

mei-41—a cell cycle checkpoint gene: Mutations in the *mei-41* gene in *D. melanogaster* were first identified on the basis of a defect in meiotic recombination (Baker and Carpenter 1972) and subsequently by their mutagen hypersensitivity (Boyd *et al.* 1976a). This latter phenotype is vividly displayed by the hypersensitivity of *mei-41* larvae to a wide range of mutagens, including ionizing radiation, UV radiation, methyl methanesulfonate, and hydroxyurea (Boyd *et al.* 1976a; Nguyen *et al.* 1979; Banga *et al.* 1986). Mutagen hypersensitivity is semidominant for strong alleles of *mei-41*, such that the dose-response curve for mutagen-induced death of females carrying a single wild-type copy of *mei-41* is inter-

mediate between those exhibited by wild-type and by *mei-41* homozygous females or hemizygous males (Boyd *et al.* 1976a; A. C. Laurencon and R. S. Hawley, unpublished data).

Mutations in *mei-41* also cause high levels of chromosome breakage and instability in mitotic cells (Baker *et al.* 1978; Gatti 1979). Neuroblast cells from *mei-41* mutant larvae show frequent chromatid and isochromatid gaps and breaks (Gatti 1979). The number of gaps and breaks is enhanced following treatment with X rays, to the extent that after 220R of irradiation, virtually all of the subsequent metaphases possess at least one break or rearrangement. The effects of *mei-41* mutations on mitotic chromosome stability are also demonstrated by genetic studies that reveal mitotic recombination and mutation during development (Baker *et al.* 1976; Baker *et al.* 1978) and high frequencies of chromosome instability in the male germline (Hawley *et al.* 1985).

The observation of chromatid gaps and breaks in the metaphase chromosomes of both mutagenized and untreated *mei-41* cells suggested that in the absence of the *mei-41* gene product, cells bearing double-strand breaks are allowed to enter mitosis. This result was surprising because many organisms possess cell-cycle checkpoint controls that prevent cells with damaged DNA from exiting G2 and entering M (reviewed in Weinert and Lydall 1993). Hari *et al.* (1995) demonstrated that *mei-41* cells fail to show an irradiation-induced delay in the entry into mitosis that is characteristic of normal cells. This result has been confirmed and extended to cells in the eye imaginal disc (M. Brodsky and G. M. Rubin, personal communication). Thus the function of the MEI-41 protein may not be in the repair of damage *per se*, but in triggering a DNA damage-dependent cell-cycle checkpoint. Activation of this checkpoint arrests the cells in G2 and prevents their entry into mitosis. This arrest serves both to prevent the suicidal effects of entering mitosis with broken chromosomes and to allow the cell time to repair that damage.

The phenotypes caused by mutations at the *mei-41* locus are reminiscent of the cellular defects exhibited in the human repair deficient syndrome ataxia telangiectasia (AT). First, like *mei-41* cells, AT cells are radiation-sensitive, and heterozygotes display a radiation-sensitivity intermediate to that observed in wild-type and homozygous cells (reviewed in Friedberg *et al.* 1995). Second, like *mei-41* cells, AT cells exhibit a high frequency of broken or rearranged chromosomes at metaphase, and X-irradiation markedly increases the number of chromosome breaks. Reduction to homozygosity for recessive markers is also common, suggesting a high rate of deletion or mitotic recombination (Bigbee *et al.* 1989). Third, among other documented anomalies in cell cycle progression, AT cells irradiated in G2 fail to display an initial block in cell cycle progression that is characteristic of normal cells (Rudolph and Latt 1989; Beamish and Lavin 1994).
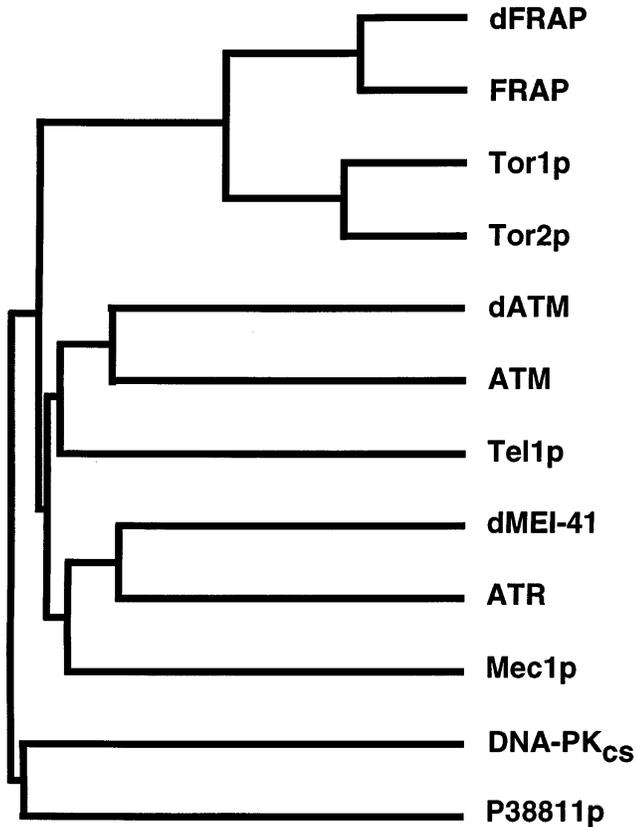
Figure 2.—Sequence relationships between members of the ATM family of proteins. To emphasize sequence-based grouping, only known members from *S. cerevisiae* (designated with a terminal p), *D. melanogaster* (indicated with an initial d), and humans are shown. Placement of the Drosophila ATM and FRAP homologs is based on limited sequencing and should be regarded as preliminary (J. J. Sekelsky and R. S. Hawley, unpublished data).

Cloning of *mei-41* and *ATM* revealed that they encoded related proteins that belong to a family of large (>250 kDa) polypeptides whose carboxy-terminal sequences are structurally similar to PI-3 kinases, although they are believed to be protein kinases (Hari *et al.* 1995; Hunter 1995; Savitsky *et al.* 1995). The *S. cerevisiae* genome encodes five members of this family; at least four members have been identified in mammals. Sequence and functional comparisons can be used to divide these into distinct subfamilies (Figure 2). The subfamily that includes ATM also includes the closely related ATR/FRP1. By sequence, ATR and ATM correspond most closely to *S. cerevisiae* Mec1p and Tel1p, respectively, and to Drosophila MEI-41 and an ATM-like gene known thus far only by sequence. Several members of this subfamily have been shown to be involved in cell cycle regulation in response to DNA damage (reviewed in Zakian 1995). A second subfamily includes the Tor1p and Tor2p proteins of *S. cerevisiae*, which have overlapping functions in cell cycle progression (Helliwell *et al.* 1994), and FKBP12-rapamycin binding pro-

tein (FRAP) from humans, also implicated in cell cycle progression (Sabatini *et al.* 1994). The final subfamily contains DNA-PK$_{cs}$, the catalytic subunit of DNA-PK. Hence, known members of the ATM family of proteins play different roles in responses to DNA damage and in regulating the cell cycle.

The roles of MEI-41 described above relate primarily to the function this protein plays in responding to DNA damage. MEI-41 also plays critical roles in at least two normal aspects of Drosophila development. The first is at the mid-blastula transition, when control of embryonic development switches from the maternal to the zygotic genome. Just prior to this critical transition, during embryonic divisions 10 through 13, the length of S phase increases dramatically as DNA replication slows, and a high level of zygotic transcription is initiated. In embryos derived from *mei-41* females, the nuclear division cycles fail to slow, high-level zygotic transcription is not initiated, and the transition to zygotic control of development at the mid-blastula transition fails (O. C. M. Sibon, A. C. Laurencon, R. S. Hawley and W. E. Theurkauf, personal communication). This is identical to the phenotype seen in embryos derived from females mutant for *grapes* (*grp*) (Sibon *et al.* 1997). Our current understanding of this process is that MEI-41 responds to the presence of incompletely replicated DNA, perhaps by binding directly to single-strand gaps, and then activates the GRP protein, a homolog of the *S. pombe* Chk1 kinase (Fogarty *et al.* 1997). GRP in turn appears to regulate the activity of TWINE (a Drosophila homolog of Cdc25) and CDC2.

MEI-41 also plays an important role during meiotic recombination. In *mei-41* females, the frequency of meiotic recombination is decreased approximately twofold (for fertile, hypomorphic alleles) (Baker and Carpenter 1972). In addition, late recombination nodules, structures that lie adjacent to synapsed meiotic chromosomes and appear to mark the sites of exchange (Carpenter 1975), are uniformly less dense than are normal recombination nodules and are associated with regions of diffuse or uncondensed chromatin (Carpenter 1979). Kleckner (1996) has argued that chromosome condensation in the area of the recombination event may be an important regulator of overall compaction along the chromosome arm. In this view, MEI-41 may both play a role in coordinating various events within the meiotic process and serve a function in controlling the relative position of exchange events. Indeed, *mei-41* oocytes exhibit a strong relaxation in the effectiveness of chiasma interference, the process that serves to keep exchanges on the same chromosome arm positioned far apart (Baker and Carpenter 1972).

MEI-41 appears to play a critical role in mediating the ability of the oocyte to monitor the progression or completion of meiotic recombination events. Although meiotic recombination in Drosophila occurs in the germarium, its effects on the meiotic cell cycle can be

observed much later, in mature stage 14 oocytes (for a review of oogenesis, see Mahowald and Kambysellis 1980). Oocytes in this stage are normally arrested at metaphase of the first meiotic division and remain so until passage through the oviduct and fertilization. This arrest is initiated by chiasmata (McKim *et al.* 1993; Jang *et al.* 1995). In recombination-defective mutants, such as *c(3)G*, *mei-218*, or *mei-9*, metaphase arrest is not achieved, so anaphase and the second meiotic division proceed. Curiously, in double mutants between *mei-41* and either *mei-9* or *mei-218*, oocytes do not enter anaphase prematurely, in spite of a predicted absence of chiasmata (J. K. Jang, K. S. McKim and R. S. Hawley, unpublished data). Double mutants between *mei-41* and *c(3)G*, however, do bypass arrest (J. J. Sekelsky, L. Messina and R. S. Hawley, unpublished data). Hence, *c(3)G* is epistatic to *mei-41* for the metaphase arrest phenotype, but *mei-41* is epistatic to *mei-9* and *mei-218*. What is the difference between *c(3)G* and *mei-9* and *mei-218*? *c(3)G* is the earliest acting recombination mutant known in Drosophila—in *c(3)G* females, all meiotic recombination (both reciprocal exchange and gene conversion) is eliminated (Carlson 1972; Hall 1972), and synaptonemal complex fails to form (Meyer, cited in Lindsley and Zimm 1992). Hence, recombination is probably never initiated in *c(3)G* mutants. In *mei-9* or *mei-218* females, however, recombination is initiated and a heteroduplex-containing intermediate is formed (Carpenter 1982). A model that incorporates these epistasis results is one in which MEI-41 monitors the status of the recombination intermediate and controls progression past metaphase if unresolved intermediates persist. In this model, progression into and through anaphase is regulated by four signals. First, upon initiation of recombination, a signal is sent that prevents anaphase. Second, resolution of recombination intermediates results in a signal that again grants permission to enter anaphase. This would require wild-type MEI-41 and may be a positive signal, or simply the inactivation or cessation of the first, inhibitory signal. Third, tension on the kinetochores, because of attempted disjuction of homologs linked by chiasmata, sends a signal that causes arrest. Fourth, passage through the oviduct sends a signal that causes meiosis to be completed. In *c(3)G* mutants, the first signal is never sent, and the absence of chiasmata prevents the third signal, so anaphase ensues; the state of MEI-41 is irrelevant. In *mei-9* or *mei-218* mutants, the first signal is sent, but counteracting this signal requires MEI-41. Hence, in the double mutants, anaphase does not occur, in spite of the absence of chiasmata, until passage through the oviduct.

The suggestion that MEI-41 may play a role in assaying the integrity of the recombination intermediate is based in part on studies of a yeast ATM family member, Mec1p. In *S. cerevisiae*, abnormal or incomplete recombination events, such as occur in mutants like *dmc1*, trigger a pachytene arrest (Bishop *et al.* 1992). Lydall *et al.*

(1996) have shown that Mec1p is required to achieve this arrest. This arrest could be similar to the lack of progression into anaphase seen in *mei-9 mei-41* or *mei-218 mei-41* double mutants. In the absence of other mutations, *mec1* mutant cells sometimes enter the first meiotic division before all recombination events are complete, indicating that Mec1p plays a crucial role in ensuring that recombination events are complete before proceeding into the meiotic divisions, as we propose for MEI-41.

As detailed above, MEI-41's role as a checkpoint protein extends beyond the sensing of DNA damage and includes the regulation of critical events in normal Drosophila development. Similar roles for MEI-41-like proteins in mammalian cells have been discussed in detail by Hawley and Friend (1996).

***mus308*—interstrand crosslink repair:** The ultimate response to DNA damage is usually to repair the damage. The strategies used depend on the type of damage: Single-strand lesions are often repaired by excision and resynthesis, whereas double-strand breaks are repaired by recombination or end-joining. Interstrand crosslinks pose a special challenge to the repair machinery, and the pathway for their removal is poorly understood. Although the other genes we discuss are of interest because of their roles in multiple pathways, the *mus308* gene is of interest because it is speicifically involved in interstrand crosslink repair, a poorly understood process in higher eukaryotes.

mus308 was identified in a large-scale screen for mutagen-sensitive mutations on the third chromosome of Drosophila (Boyd *et al.* 1981) and was unique among the 11 complementation groups identified in that it was the only gene that when mutated caused sensitivity to the crosslinking agent nitrogen mustard, but not to noncrosslinking mutagens such as methylmethane sulfonate and UV light. Indeed, *mus308* remains unique among more than 30 known Drosophila mutagen-sensitive loci in its specific sensitivity to crosslinking agents and shares this unusual phenotype with only one other known mutation, the *snm1* gene of *S. cerevisiae*, which encodes a protein of unknown function. In the absence of mutagen, no obvious phenotype is associated with loss of *mus308* function. The implication of these results is that at least one step in the pathway by which interstrand crosslinks are repaired in Drosophila is carried out by a protein not essential for other known repair pathways such as nucleotide excision repair, although components of the nucleotide excision repair (NER) pathway likely play a role in crosslink repair as discussed below.

The mechanism of interstrand crosslink repair has been best characterized in *E. coli*, where genetic evidence implicates both the nucleotide excision and recombinational repair pathways in the process (reviewed in Friedberg *et al.* 1995). Elegant biochemical studies of psoralen-crosslinked substrates have reproduced several of the steps in this process *in vitro* (Sladek *et al.* 1989).

The initial step in this pathway is incision by the UvrABC endonuclease complex, which cleaves to either side of the crosslinked nucleotide on one strand. It is hypothesized that DNA polymerase I, by virtue of its 5′-3′ exonuclease activity, then extends the nick at the 3′ side of the crosslink into a gap. The single-stranded DNA thus exposed provides a template for RecA-mediated recombination with the homologous chromosome (repair can occur only in cells that have undergone at least partial replication of their genome). Branch migration of the Holliday structure thus formed across the location of the crosslink results in displacement of the still covalently linked oligonucleotide resulting from the initial endonucleolytic cleavages, leading to a transient three-stranded structure. Resolution of the recombinational intermediates leaves one homologous chromosome with a gap, which can be filled *in vivo* by any of the three *E. coli* DNA polymerases but which is most likely repaired by Pol I. The other homolog is left with an oligonucleotide adduct on an otherwise normal duplex, which can be excised by the UvrABC endonuclease and processed by the standard excision repair pathway.

The initial steps in interstrand crosslink repair in Drosophila and other higher eukaryotes are likely also carried out by the enzymes of the NER pathway. However, surprising results from Bessho *et al.* (1997) have indicated that extracts from rodent cells, as well as purified reconstituted human excision nuclease, cleave adjacent to interstrand crosslinks, but they do not cleave to either side of the crosslink, as in *E. coli*. Rather, they make two incisions 22–26 nucleotides apart that are both located 5′ of the crosslink. Thus, models for subsequent steps in this pathway must take into account the failure of these initial incisions to release the covalent linkage between the two crosslinked strands, an event that must be accomplished at some stage to permit recombinational repair to proceed. Bessho *et al.* (1997) propose several alternative models to explain how the crosslink might be ultimately removed; however, the precise molecular nature of this pathway remains to be determined.

There is no direct evidence to indicate whether the crosslink repair pathway in Drosophila more closely resembles that in *E. coli* or in humans. Characterization of the *mus308* gene has revealed the existence of a unique polypeptide, absent from both *E. coli* and *S. cerevisiae*, that is essential for crosslink repair in Drosophila. The *mus308* gene encodes a remarkable protein ideally suited for a role in recombinational repair (Harris *et al.* 1996). The MUS308 polypeptide is unique among known proteins in that it includes an amino-terminal domain with homology to the large superfamily 2 of DNA helicases and a carboxy-terminal domain closely related to the polymerase domain of *E. coli* DNA polymerase I. MUS308 is the first reported protein with both helicase and polymerase domains in a single polypeptide and is also the first reported eukaryotic homolog of DNA polymerase I. Although the precise role of this protein in crosslink repair

remains to be elucidated, both helicase and polymerase activities are required at several steps in extant models of recombinational repair. It will be of particular interest to determine the relevance of the physical linkage between the helicase and polymerase domains to this process. One possibility might be energetic coupling of repair synthesis with branch migration across the region containing the crosslink, necessitated perhaps by the unusual barrier to branch migration presented by the crosslink. Conservation of this juxtaposition of helicase and polymerase motifs in a *Caenorhabditis elegans* homolog of *mus308* (Harris *et al.* 1996) supports the functional significance of this arrangement of domains, and preliminary evidence suggests that a similar polypeptide may be encoded in the human genome (P. Harris and K. C. Burtis, unpublished data). However, a genetic function for the worm and human homologs in DNA repair remains to be demonstrated, and further biochemical studies of MUS308 will be necessary to determine its *in vitro* enzymatic characteristics and their *in vivo* function.

In light of the multiple roles of the other DNA repair proteins discussed in this review, it is interesting to speculate on the biological roles that might be played by *mus308*. It seems likely that interstrand crosslinks are a relatively rare variety of genetic lesion. However, a more common occurrence that would call upon a similar pathway of DNA repair is the bypass of unrepaired lesions on the template strand during DNA replication, resulting in gaps in the daughter strand opposite a damaged template. As with interstrand crosslinks, accurate genetic information can be obtained in this situation only by some form of recombinational repair. The hypermutability of *mus308* mutants when exposed to mutagens such as N-ethyl-N-nitrosourea is consistent with a general role for *mus308* in postreplicational recombinational repair of such lesions, as suggested by Aguirrezabalaga *et al.* (1995). Thus, interstrand crosslink repair may represent only a minor (albeit essential) function of the MUS308 protein in DNA repair *in vivo*. The nonessential nature of this protein in the absence of crosslinking mutagens may simply reflect the presence of redundant pathways (such as error-prone translesion synthesis) for repair of gaps opposite damaged templates.

mus209—PCNA, DNA replication fidelity, and position-effect variegation: Proliferating cell nuclear antigen (PCNA) is an important component of the DNA replication machinery (reviewed in Stillman 1994). This protein forms a sliding clamp around the replication fork and contributes to polymerase processivity and to coordinating replication on the leading strand with that on the lagging strand. PCNA is also required for some repair pathways, including NER (Nichols and Sancar 1992; Shivji *et al.* 1992) and mismatch repair (MMR; Umar *et al.* 1996). Although the function of PCNA in NER is during the synthesis step and therefore is likely to be similar to its function in replication, at

least part of the requirement in MMR is believed to occur before the synthesis step.

In Drosophila, PCNA is encoded by the *mus209* gene (Henderson *et al.* 1994). Although null mutations in *mus209* are lethal in essentially all cells, alleles for which the gene is named are homozygous viable at normal temperatures, but confer both sensitivity to mutagens (likely related to the roles of PCNA in repair pathways such as NER and MMR) and female sterility (perhaps because of an embryonic requirement for maternally loaded protein). Genetic analysis of temperature-sensitive *mus209* mutations revealed an interesting additional phenotype: suppression of position-effect variegation (PEV).

PEV is the mosaic inactivation of a normally euchromatic gene when it is placed near heterochromatin (or a normally heterochromatic gene when it is removed from heterochromatin). Several models have been proposed to account for the action of heterochromatin on gene expression (reviewed in Weiler and Wakimoto 1995). Although some of these suggest that differences in copy number may mediate mosaic expression and therefore may explain the effects of PCNA mutants on PEV in terms of its known functions in replication, available data indicate that variegating genes are not under-represented in diploid tissues (Karpen and Spradling 1990; Wallrath *et al.* 1996). The most viable models at present attribute the effects of heterochromatin on gene expression to subnuclear location (Wakimoto and Hearn 1990; Talbert *et al.* 1994; Dernburg *et al.* 1996) or to changes in chromatin structure (Wallrath and Elgin 1995). It is not immediately obvious what role a replication protein would play in such models. One trivial possibility is that *mus209* mutants are delayed in development and that this delay contributes to suppression of PEV, in a manner similar to decreasing the temperature during development. However, the recent report of a genetic interaction between *mus209* and *Cramped*, a member of the *Polycomb-Group* of genes, and a possible physical interaction between the proteins, suggests that PCNA may indeed have a specific function in gene silencing (Yamamoto *et al.* 1997). Elucidation of this function should shed new light on the functions of this remarkable protein.

***mei-9*—nucleotide excision repair meets meiotic recombination:** Meiotic recombination is used to ensure the segregation of homologous chromosomes from one another at the first meiotic anaphase. The process is best understood at a molecular level in *S. cerevisiae* (for reviews see Kleckner 1996; Stahl 1996). In this organism, meiotic recombination is proposed to proceed via a pathway involving: (1) formation of a double-strand break on one chromosome; (2) resection of the ends by a 5′-3′ exonuclease to yield 3′ single-stranded tails; (3) invasion of a homologous sequence, usually the homologous chromosome, and extension by new synthesis; (4) ligation to yield a double-Holliday junction (HJ) intermediate; and (5) resolution to produce crossovers or noncrossovers. Although many of the details remain unclear, several of the proposed intermediates, including double-strand breaks before and after resection, and double-HJ structures, have been detected in physical assays (Sun *et al.* 1989; Cao *et al.* 1990; Collins and Newlon 1994; Schwacha and Kleckner 1995).

An important clue leading to the initial proposal of recombination initiated by a double-strand break was the discovery that many radiation-sensitive mutations, especially those in the *RAD52* epistasis group, are also defective in meiotic recombination (Prakash *et al.* 1980; Szostak *et al.* 1983). A similar relationship between meiotic recombination and somatic DNA metabolism had been shown previously in Drosophila. Baker *et al.* (1976) found increased levels of mitotic recombination and chromosome breakage, both spontaneous and induced, in the presence of mutations that decrease meiotic recombination. Boyd *et al.* (1976a) screened for mutations that conferred heightened sensitivity to mutagens and found some to be allelic to mutations that cause defects in meiotic recombination, including *mei-9* and *mei-41*.

Mutations in *mei-9* result in a severe decrease in the level of meiotic crossing over, as well as increased sensitivity to methyl methanesulfonate, ultraviolet light, and ionizing radiation (Baker and Carpenter 1972; Boyd *et al.* 1976a). Subsequent studies showed that *mei-9* is required for the incision step in NER (Boyd *et al.* 1976b). *mei-9* encodes the Drosophila homolog of *S. cerevisiae* Rad1p (Sekelsky *et al.* 1995), an NER protein not required for meiotic recombination (Snow 1968; Game *et al.* 1980), except under very special conditions (Resnick *et al.* 1983; Kirkpatrick and Petes 1997). Hence, the meiotic recombination pathways in Drosophila and Saccharomyces differ in at least the *mei-9*-dependent step.

What is the role of *mei-9* in meiotic recombination? Suggestions come from an examination of the mutant phenotype coupled with an understanding of the biochemical functions of the yeast and mammalian homolog. In females homozygous for *mei-9* mutations, crossing over is reduced about 20-fold (Baker and Carpenter 1972). That the observed reduction is uniform in all genetic intervals suggested to Baker and Carpenter (1972) that *mei-9* is required for the process of exchange itself. In contrast to the drastic reduction in reciprocal recombination, meiotic gene conversion is not reduced in *mei-9* females (Carpenter 1982). However, convertant progeny are recovered as individuals mosaic for two maternal alleles, a condition termed postmeiotic segregation (PMS) or half-conversion. Thus, meiotic recombination in Drosophila involves the formation of an intermediate containing heteroduplex DNA. Although *mei-9* females can produce noncrossovers from this intermediate, they can neither repair mismatches within heteroduplex, nor produce crossovers.

The known biochemical functions of MEI-9 homologs suggest functions for MEI-9 that help us to understand
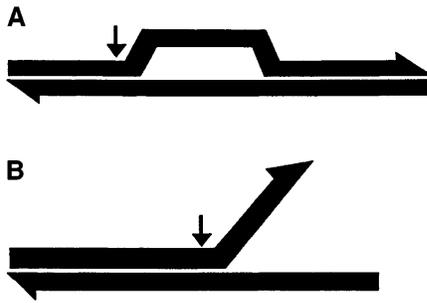
Figure 3.—Structures cleaved by the Rad1p/Rad10p and XPF/ERCC1 endonucleases *in vitro*. These junction-specific endonucleases cut at 5′ double-stranded to 3′ single-stranded junctions. (A) Bubbles mimic intermediates in nucleotide excision repair, in which these proteins nick 5′ to the site of damage. *In vitro*, the bottom strand would also be nicked, but in NER, the undamaged strand is protected. (B) Flaps mimic intermediates believed to occur in some types of double-strand break repair, such as single-strand annealing (Fishman-Lobell and Haber 1992).

this phenotype. Rad1p, together with Rad10p, is a DNA structure-specific endonuclease (Tomkinson *et al.* 1993; Bardwell *et al.* 1994) (Figure 3), as are their mammalian homologs XPF/ERCC4 and ERCC1 (Park *et al.* 1995; Sijbers *et al.* 1996). This suggests that the function of MEI-9 in meiotic recombination may be to cut HJs within recombination intermediates. This proposal seems to be at odds with the report that the Rad1p/Rad10p endonuclease does not cut HJs *in vitro* (Davies *et al.* 1995; but see Habraken *et al.* 1994). However, it seems reasonable to suppose that HJ conformation in the context of the multiprotein recombination nodule may be quite different from HJ structure *in vitro*. Alternatively, MEI-9 may simply differ from Rad1p in the constellation of structures it can cleave, or its activity may be modulated or augmented by other components of the recombination nodule.

If MEI-9 is involved in resolving HJs, how is it that crossover resolution is specifically eliminated, whereas noncrossover resolution is allowed? In many HJ-based models for recombination, both crossovers and noncrossovers have been presumed to require nicking of two strands at the HJ, followed by exchange and religation with one another (Holliday 1964; Meselson and Radding 1975; Szostak *et al.* 1983) (Figure 4A). However, alternative proposals for resolution of the double-HJ intermediates observed in yeast have been presented. Although we do not know the structure of the recombination intermediate acted upon by MEI-9, these alternative resolution models can explain aspects of the *mei-9* mutant phenotype not easily accommodated by other models, so we will limit the discussion below to consideration of double-HJ intermediates.

Two features of the *mei-9* mutant phenotype must be incorporated in models for the MEI-9 function: inability to generate crossovers and the presence of PMS. Al-

though these may represent two separable functions for MEI-9, a more parsimonious view is that both phenotypes are the result of a single biochemical defect. A model that accommodates this view is one that requires that heteroduplex mismatch correction depends on strand nicking. In the model shown in Figure 4B, nicks at one HJ are used both to effect repair of mismatches and to remove the second HJ, by branch migration off the nicked ends (or, for example, by exonucleolytic degradation from the nicks to the HJ).

If MEI-9 is required to make nicks such as proposed above, we are still left to explain the ability to generate noncrossovers. In principle, a double-HJ structure could be resolved without cutting either junction, by branch migration of the two junctions toward one another, followed by topoisomerase-mediated decatenation (Figure 4B) (Thaler *et al.* 1987). Gilbertson and Stahl (1996) conducted a clever test of DSB models in yeast and made observations consistent with the existence of such a noncutting pathway. A similar pathway in Drosophila could provide a mechanism for removing double-HJ structures that have not been cleaved by the appropriate endonuclease (MEI-9?).

Resolution by topoisomerases can produce only non-crossover products. If mismatch correction requires nicking, as discussed above, then the usual mode for generating noncrossovers should also involve nicking, because PMS is not normally observed among either crossovers or noncrossovers (Chovnick *et al.* 1971). Hence, a topoisomerase-mediated resolution pathway would represent only a backup pathway for dealing with recombination intermediates that persist beyond the normal time for resolution. Alternatively, a secondary pathway for removal of mismatches may exist but be defective in *mei-9* females. It is also possible that the PMS observed in *mei-9* mutants is a consequence of inappropriate formation of mismatches, as opposed to defective correction of existing mismatches. Mismatches are not observed in yeast double-HJ intermediates until the time of resolution (Schwacha and Kleckner 1995). If this is true for Drosophila, then perhaps the defect in *mei-9* mutants is the formation of mismatches via the *mei-9*-independent resolution pathway, such as during branch migration of the two HJs in anticipation of topoisomerase-mediated unwinding.

The models described above encompass only a subset of the possibilities that include double-HJ intermediates. As noted, we have little information regarding either the nature of the recombination intermediate in Drosophila or its mode(s) of resolution. Although the models as diagrammed make clear predictions regarding the arrangements of heteroduplex DNA on the recombinant products, this information is typically lost to the experimentalist, because of mismatch correction activities and, in metazoans, the inability to recover more than one of the four products of a meiosis. The absence of mismatch repair activities in *mei-9* mutants
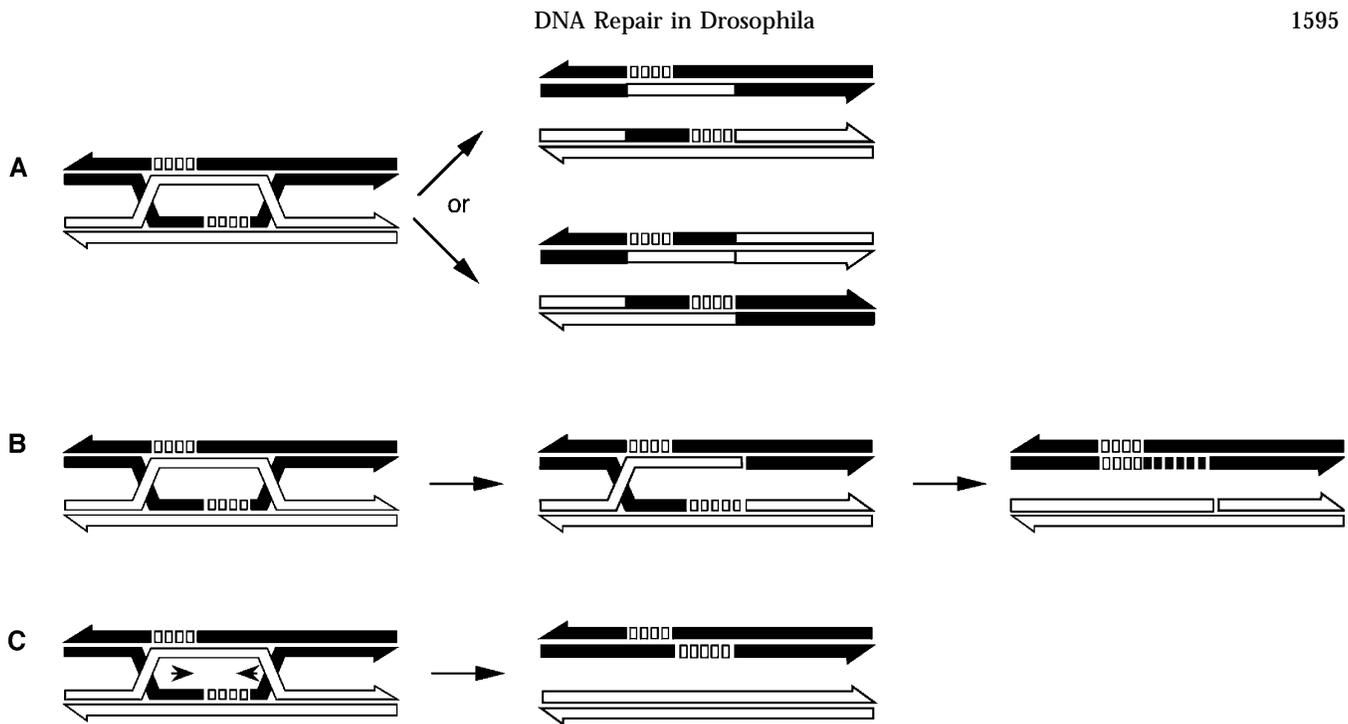
Figure 4.—Models for resolution of double-HJ intermediates. A possible intermediate resulting from double-strand break meiotic recombination is shown at the left of each panel. White and black represent homologous, nonsister chromatids; dashed lines (□□□□) signify new synthesis occurring during recombination. The structure diagrammed is one possible intermediate; the actual intermediate may differ in several ways: First, the two sides of the DSB may be asymmetrically processed so that heteroduplex DNA is asymmetrical. Second, mismatches may be eliminated at an early stage so that none exist in the intermediate. Third, branch migration of one or both HJs can remove or create additional heteroduplex DNA. (A) Resolution by cutting both HJs. The relative orientations of the cuts determine whether a crossover or noncrossover is produced. The examples shown are for both being cut in the "horizontal" mode (strands depicted as crossing) to produce a noncrossover (upper) and for the left HJ being cut in the "horizontal mode" and the right in the "vertical" mode to produce a crossover (lower). (B) Resolution by nicking only one HJ. The remaining junction is removed by branch migration off the nicked ends or by exonucleolytic activity. In this model, the nicks made at the first HJ are also used to direct mismatch correction. (C) Resolution mediated by branch migration and topoisomerase, without strand nicking, to produce noncrossovers.

provides a unique opportunity to detect heteroduplex within recombination intermediates in Drosophila and therefore to learn about the structure of the intermediate and its resolution. Half-tetrad analysis (in which two of four chromatids are recovered) in *mei-9* mutants should provide a wealth of information regarding not only the role of MEI-9 in recombination, but also the nature of the recombination pathway.

CONCLUSIONS

Responses to DNA damage include the detection of the damage, arrest of the cell cycle, and DNA repair. We have discussed Drosophila genes required for each of these processes. Studies of the effects of mutations in these genes show that they are required not only for pathways known to be conserved between yeast, flies, and mammals, but also for other important cellular DNA metabolism processes, which in some cases might also be conserved. Although it is certainly not the case that examination of each DNA repair gene in Drosophila will provide such insights, it is clear that this organism has much to offer as a model for genetic studies of DNA repair.

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