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Multiple Functions of Drosophila BLM Helicase in Maintenance of Genome Stability

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ABSTRACT

Bloom Syndrome, a rare human disorder characterized by genomic instability and predisposition to cancer, is caused by mutation of *BLM*, which encodes a RecQ-family DNA helicase. The *Drosophila melanogaster* ortholog of BLM, DmBlm, is encoded by *mus309*. Mutations in *mus309* cause hypersensitivity to DNA-damaging agents, female sterility, and defects in repairing double-strand breaks (DSBs). To better understand these phenotypes, we isolated novel *mus309* alleles. Mutations that delete the N terminus of DmBlm, but not the helicase domain, have DSB repair defects as severe as those caused by null mutations. We found that female sterility is due to a requirement for DmBlm in early embryonic cell cycles; embryos lacking maternally derived DmBlm have anaphase bridges and other mitotic defects. These defects were less severe for the N-terminal deletion alleles, so we used one of these mutations to assay meiotic recombination. Crossovers were decreased to about half the normal rate, and the remaining crossovers were evenly distributed along the chromosome. We also found that spontaneous mitotic crossovers are increased by several orders of magnitude in *mus309* mutants. These results demonstrate that DmBlm functions in multiple cellular contexts to promote genome stability.

BLM is an ATP-dependent helicase that belongs to the RecQ family (Ellis *et al.* 1995). Mutations in *BLM* cause Bloom Syndrome (BS), a rare, autosomal recessive disorder characterized by proportional dwarfism, sterility, and immunodeficiency. BS patients have an increased incidence of many types of cancers, including leukemias, lymphomas, and carcinomas. BS cell lines are genomically unstable, showing a high rate of chromosome breaks and rearrangements and increased exchange between sister chromatids and homologous chromosomes (Chaganti *et al.* 1974; German *et al.* 1977).

In vitro, the human BLM protein acts on structures mimicking those formed during DNA replication and recombination. It promotes branch migration of Holliday junctions (HJs) and unwinds HJs and D-loops (Karow *et al.* 2000; van Brabant *et al.* 2000; Bachrati *et al.* 2006). Biochemical assays have also revealed a strand-annealing activity that may act in conjunction with its helicase activity (Cheok *et al.* 2005; Machwe *et al.* 2005). Together, these activities suggest that BLM may function during DNA replication, DNA repair, and/or meiotic recombination. The exact roles that BLM plays in these multiple contexts are currently the subject of intense investigation.

Accumulating evidence suggests that BLM plays an important role in the recovery of damaged and/or stalled replication forks. BLM accumulates at sites of stalled replication forks, where it interacts with repair and checkpoint proteins, including p53, 53BP1, and Chk1 (Sengupta *et al.* 2003, 2004). In addition, *in vitro* studies have shown that BLM can regress a stalled or collapsed replication fork in such a way that the damage or blockage can be bypassed (Ralf *et al.* 2006).

Other studies suggest that BLM also acts during the repair of DNA double-strand breaks (DSBs). BLM interacts with the homologous recombination repair proteins Rad51, Mlh1, and replication protein A via its N and C termini (Brosh *et al.* 2000; Pedrazzi *et al.* 2001; Wu *et al.* 2001). These interactions, viewed in light of the increased crossover phenotype seen both in BS cells and in embryonic stem cells of BLM knockout mice, are consistent with BLM acting within one or more repair pathways that do not result in crossovers (Chester *et al.* 1998; Hu *et al.* 2005).

BLM may also function in meiotic recombination, but its role in this process is not well understood. In mouse spermatocytes, BLM foci associate with the synaptonemal complex and often colocalize with the recombination proteins RPA, Rad51, and Dmc1 (Walpita *et al.* 1999; Moens *et al.* 2000). Mutations in *SGS1*, which

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encodes the sole RecQ helicase in *Saccharomyces cerevisiae*, have variable effects on meiotic recombination. Two reports showed no meiotic defects in *sgs1* mutants lacking helicase activity (Watt *et al.* 1996; Miyajima *et al.* 2000), while other studies showed a modest increase in meiotic crossovers in *sgs1* mutants, suggesting a role for Sgs1 in regulating resolution of recombination intermediates (Rockmill *et al.* 2003; Jessop *et al.* 2006).

To learn more about BLM functions, we characterized novel alleles of the Drosophila *mus309* gene, which encodes DmBlm (Kusano *et al.* 2001). Previous studies have used two alleles, one a nonsense mutation and the other a missense mutation, either in *trans* to one another or hemizygous. These mutants have reduced fertility, increased sensitivity to alkylating agents and ionizing radiation (IR), and defects in repair of DSBs generated by excision of transposable elements (Boyd *et al.* 1981; Beall and Rio 1996; Kooistra *et al.* 1999; Kusano *et al.* 2001; Adams *et al.* 2003; McVey *et al.* 2004b). We generated deletion alleles predicted to remove either the N terminus or both the N terminus and the helicase domain. Through genetic characterization of these and previously existing mutations, we found important roles for DmBlm in early embryogenesis and meiotic recombination. We also report that *mus309* mutants, like *S. cerevisiae sgs1* mutants and human BS cells, have elevated rates of mitotic crossing over associated with DSB repair. We discuss possible functions of DmBlm in these processes.

MATERIALS AND METHODS

Deletion alleles of *mus309*: Deletion alleles of *mus309*, which is located on chromosome *3* in cytological region 86E17, were generated by *P*-element excision (reviewed in Adams and Sekelsky 2002). *P{EPgy2}mus309^{EY03745}* harbors a *P* element just upstream of the ATG corresponding to the initiator codon (Bellen *et al.* 2004). Flies homozygous for *P{EPgy2}mus309^{EY03745}* are viable and fertile, and we did not detect any defects like those of *mus309* mutations in these homozygotes (data not shown). A total of 759 excisions were screened by PCR to detect any that created deletions in *mus309* protein-coding sequences but not in the other direction. Final structures were determined by DNA sequencing. The four deletion alleles that we recovered are named *mus309^{N1}*, *mus309^{N2}*, *mus309^{N3}*, and *mus309^{N4}*.

Ionizing radiation sensitivity assay: Balanced, heterozygous parents were crossed and allowed to lay eggs on grape-juice agar plates for 12 hr. Embryos were allowed to develop at 25° until larvae reached third instar stage. Plates were then irradiated in a Gammator 50 irradiator at a dose rate of 225 rad/min, after which larvae were transferred to bottles. Relative survival was calculated as the number of mutant adults (homozygous or heteroallelic) divided by the total number of adults (mutant and heterozygous) that eclosed within 10 days of irradiation. Ratios were normalized to an unirradiated control. In experiments with crosses of heterozygous females to wild-type males, there was no difference in survival of heterozygous progeny relative to wild-type progeny (data not shown), indicating that *mus309* is completely recessive for IR sensitivity.

***P{w^a}* assay:** Repair of DNA double-strand gaps was measured after excision of the *P{w^a}* transposable element (on the *X* chromosome and conferring apricot-colored eyes), as described previously (Adams *et al.* 2003; McVey *et al.* 2004a,b,c). Single *y w P{w^a}/Y; +/CyO, Δ2-3; mus309^a/mus309^b* males were crossed to homozygous *y w P{w^a}* females. Excision and repair that occurs in the male germline is assayed by scoring daughters that do not inherit the *CyO, Δ2-3* chromosome. The frequency of *P{w^a}* excision can vary in different genetic backgrounds (McVey *et al.* 2004b; LaRocque *et al.* 2007). Enzymatic induction of site-specific DSBs at a high frequency can bias recovery of repair products. In cases where both sister chromatids are cut, the lack of a homologous template favors repair through nonhomologous end joining (NHEJ). If the enzyme persists through multiple cell cycles, then repeated DSB formation and repair favors recovery of products that can no longer be cut. In the experiments reported here, we estimate that 10–20% of all progeny of males with *P{w^a}* and transposase are derived from cells in which the element has been excised and the gap repaired. This low level of excision reduces the biases described above. Within this range of excision rates, the ratios of different types of repair products do not differ significantly (LaRocque *et al.* 2007). To normalize for differences in excision rate, we determined the fraction of progeny known to be derived from excision and repair (those with red or yellow eyes, as described below) in which repair occurred through completed synthesis-dependent strand annealing (SDSA; those with red eyes, which come from SDSA with annealing of LTRs, as shown in supplemental Figure S1 at http://www.genetics.org/supplemental/). This calculation is an underestimate, since completed SDSA can also restore the entire 14-kb *P{w^a}* element. The apricot-eyed progeny that result from complete restoration cannot be distinguished from apricot-eyed progeny derived from cells in which the *P{w^a}* was never excised, so they are not included in our estimate of completed SDSA. On the basis of analysis of mutants that cannot carry out early steps in homologous recombination, we estimate that completion of SDSA such that the entire *P{w^a}* is restored is about as frequent as completion of SDSA through annealing of LTRs (Adams *et al.* 2003; McVey *et al.* 2004b).

For each genotype assayed, we counted progeny from multiple vials, each with a single male parent (supplemental Table S4 at http://www.genetics.org/supplemental/). Because excision and repair is predominantly premeiotic, we treated each vial as a separate experiment. Statistical comparisons were done for each pair of genotypes, using a Mann–Whitney *U*-test done with InStat 3 (GraphPad). *P*-values are reported in supplemental Table S5.

Synthesis tract lengths and occurrence of deletions were assayed as described previously (Adams *et al.* 2003; McVey *et al.* 2004b). A single yellow-eyed female was taken from each vial, thereby ensuring that they represent independent repair events, and mated to *FM7, w^1* males (Sekelsky *et al.* 1999). DNA was isolated from white-eyed male progeny. The absence of white-eyed males indicated a male-lethal repair event, and in this case DNA was prepared from white-eyed daughters. DNA was amplified by PCR to determine which repair events had synthesis tracts that extended at least 5, 920, 2420, and 4674 bp from the cut site at the 3′-end of the *P* element (supplemental Table S6 at http://www.genetics.org/supplemental/). For those that lacked at least 5 bp of synthesis, additional PCR was done to detect deletions from the 3′-end of the *P* element.

Crossover and nondisjunction assays: To measure premeiotic crossovers in the male germline, virgins of the genotype *st mus309^{D2} e/TM6B* were mated to males that were wild type or

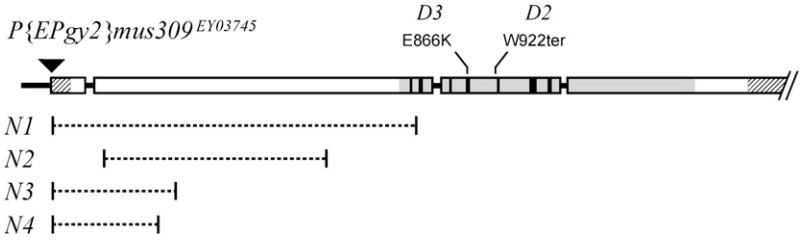

FIGURE 1.—*mus309* alleles. Boxes indicate exons; untranslated regions are hatched (only the beginning of the 3′-UTR is shown) and the region encoding the RecQ core is shaded. Vertical lines mark the positions of the seven conserved motifs of superfamily II helicases. The insertion site of the *P{EPgy2}mus309*^{EY03745} element used to generate deletions is indicated by a solid triangle. The positions of the nonsense mutation in *mus309*^{D2} and the missense mutation in *mus309*^{D3} are given above the schematic, and the regions deleted in *mus309*^{N} alleles are indicated below with dashed lines.

that carried another allele of *mus309*. Embryos were collected and allowed to hatch, and third instar larvae were irradiated and transferred to bottles. Adult males were collected after eclosion and crossed to *ru h st ry e* virgin females. Progeny were scored as parental or recombinant with respect to *st* and *e*. Data were analyzed as described for the *P{w*^{a}*}* assay, with each vial being considered a different experiment.

To measure meiotic crossovers, females with the genotype *net dpp*$^{d\text{-}ho}$ *dp wg*$^{Sp\text{-}1}$ *b pr cn*/+; *mus309*^{N2} were crossed to *net dpp*$^{d\text{-}ho}$ *dp b pr cn* males, and the progeny were scored for all visible markers. Meiotic nondisjunction (ndj) of the *X* chromosomes was measured by crossing *y w*; *mus309*^{N2} (or *mus309*^{N2}/*mus309*^{D2}) females to *y cv v f*/*Dp(1;Y)B*^{S} males. The duplication on the *Y* chromosome carries a dominant mutation causing bar-shaped eyes. Normal progeny are females whose eyes are red and non-Bar and males whose eyes are white and Bar. Diplo-*X* ova give rise to *XXY* females (and *XXX* progeny, which do not survive) whose eyes are white and Bar. Nullo-*X* ova give rise to *XO* males (and *YO* progeny, which do not survive) whose eyes are vermillion and non-Bar (these males have the additional patroclinous phenotypes of crossvein-less wings and forked bristles). *X* ndj is calculated as the percentage of progeny that arose from ndj (Bar females and non-Bar males), after correcting for loss of half of the diplo-*X* ova and half of the nullo-*X* ova. Crosses were set up as five females and three males/vial. For Table 2, data were pooled from a total of 15 vials/genotype. For each genotype, mean ndj frequency per vial was similar to the valued determined from pooling progeny counts.

Studies of embryonic development: Virgin females of various genotypes were mated to *w*^{1118} males. Eggs were collected on grape-juice agar plates for 12 hr and scored for hatching 48 hr later. To analyze syncytial-stage nuclear divisions, embryos were collected for 2 hr on grape-juice plates, dechorionated with 50% bleach, devitellenized with heptane, and fixed in 3.7% formaldehyde (Fisher F79-500). Fixed embryos were stained with 1 μg/ml DAPI and mounted with Fluoromount-G (SouthernBiotech). Images were taken with WinView/32 software (Roper Scientific) on a Nikon Eclipse E800 fluorescence microscope. To score cellularization and gastrulation (germband extension), embryos were collected for 2 hr, aged for 4 hr, and processed in the same way.

RESULTS

Isolation of new *mus309* mutant alleles: The DmBlm protein, encoded by *mus309*, is 1487 amino acid residues and, like human BLM and yeast Sgs1p, contains a DEAH-box helicase domain with seven conserved motifs, a RecQ family C-terminal domain, and a helicase RNase D C-terminal domain (Figure 1). Three mutant alleles of *mus309* have been described previously (BOYD *et al.* 1981), two of which, *mus309*^{D2} and *mus309*^{D3}, are still available. The chromosomes carrying these mutations were homozygous viable when originally isolated, but are now homozygous lethal, presumably due to second-site mutations that arose in the stocks. Consequently, most genetic studies have used heteroallelic (*mus309*^{D2}/*mus309*^{D3}) or hemizygous genotypes. The *mus309*^{D2} mutation creates a premature stop codon between the regions encoding helicase motifs III and IV (KUSANO *et al.* 2001). We carried out RT–PCR using primers that span the second intron and RNA isolated from adults hemizygous for *mus309*^{D2} and were unable to detect a product (data not shown), suggesting that any transcript produced is degraded through nonsense-mediated decay. The *mus309*^{D3} allele is a missense mutation that changes the glutamic acid residue in the conserved DEAH motif to lysine (KUSANO *et al.* 2001). This motif is critical for nucleotide cofactor binding and hydrolysis, so any DmBlm protein produced by this allele is predicted to lack helicase activity.

To isolate additional alleles of *mus309* in a common genetic background, we conducted a *P*-element excision screen. We used *P{EPgy2}mus309*^{EY03745}, which is an insertion of a *P* element into sequences corresponding to the 5′-UTR of *mus309*. After inducing excision, we obtained four new alleles that delete various amounts of the 5′-end of *mus309*; we named these alleles *mus309*^{N1}, *mus309*^{N2}, *mus309*^{N3}, and *mus309*^{N4} (Figure 1). Each of these alleles retains an intact promoter, and RT–PCR using primers that flank the second intron demonstrates that truncated transcripts are present at approximately wild-type levels (data not shown).

The *mus309*^{N1} deletion removes 2480 bp, including the start codon and sequences encoding part of the helicase domain. The first in-frame AUG is at codon 813, so any protein produced by this allele would lack helicase motifs I and Ia. This allele, like the nonsense allele *mus309*^{D2}, appears to be genetically null (see below). The other deletions (*mus309*^{N2}, *mus309*^{N3}, and *mus309*^{N4}) do not extend into sequences encoding the helicase domain. In *mus309*^{N3} and *mus309*^{N4}, the start codon is deleted. Initiation at the first in-frame AUG

TABLE 1

Hatch rates and staging of embryonic lethality

Genotype	Hatched (*n*)	Cellularized	Gastrulated
+/+	98 (588)	99	96
D2/D3	6.8 (931)	ND	ND
D3/D2	5.9 (593)	ND	ND
N1/N1	2.7 (1057)	ND	ND
N1/D2	4.0 (417)	40	0
N1/D3	9.2 (454)	ND	ND
N2/N2	38 (1120)	76	59
N3/N3	42 (1805)	ND	ND
N4/N4	36 (987)	ND	ND

mus309 alleles of mothers are listed, with the maternal allele at the left of the slash. All values are percentages except those in parentheses, which indicate the number of embryos scored for hatching. For cellularization and gastrulation, $n = 100$ for each genotype. ND, not determined.

would yield a protein lacking the N-terminal 236 residues. The deletion in *mus309*^{N2} is unusual in that it begins downstream of the site of the *P{EPgy2}* insertion. We speculate that this deletion arose after an initial transposition of the element to this position in the flies carrying this element and transposase. The deletion begins after the start codon, but results in a frameshift. There is an AUG in the 5′-UTR that is in the correct reading frame for translation through the helicase domain. Initiation at this AUG would produce a protein with 35 residues of novel sequence joined to DmBlm residue 567. Residues 567 and 568 are both methionine, so it is also possible that translation may start at either of these sites or farther downstream. Regardless of the start position, DmBlm produced by the N-terminal truncation alleles lacks at least 236 residues in the case of *mus309*^{N3} and *mus309*^{N4} and at least 566 residues in the case of *mus309*^{N2}.

Female sterility in *mus309* mutants is due to maternal-effect embryonic lethality: Previous studies have shown that fertility is greatly reduced in *mus309* mutant females (Boyd *et al.* 1981; Beall and Rio 1996; Kusano *et al.* 2001). This could result from defects in meiosis or oogenesis or from a requirement for DmBlm during early embryogenesis. Females mutant for *mus309* laid morphologically normal eggs at a frequency similar to that of wild-type females (data not shown); however, embryos from females carrying mutations that disrupt the helicase domain (*mus309*^{D2}, *mus309*^{D3}, and *mus309*^{N1}) had extremely low hatch rates (Table 1). To gain insight into the cause of the embryonic lethality, we examined embryos fixed at various stages of development. In embryos fixed during syncytial nuclear divisions, there were frequent anaphase bridges, asynchronous mitoses, and gaps in the normally uniform monolayer of nuclei; most embryos had at least one visible defect (Figure 2). Hatch rates among embryos from females carrying N-terminal deletions that do not include the helicase

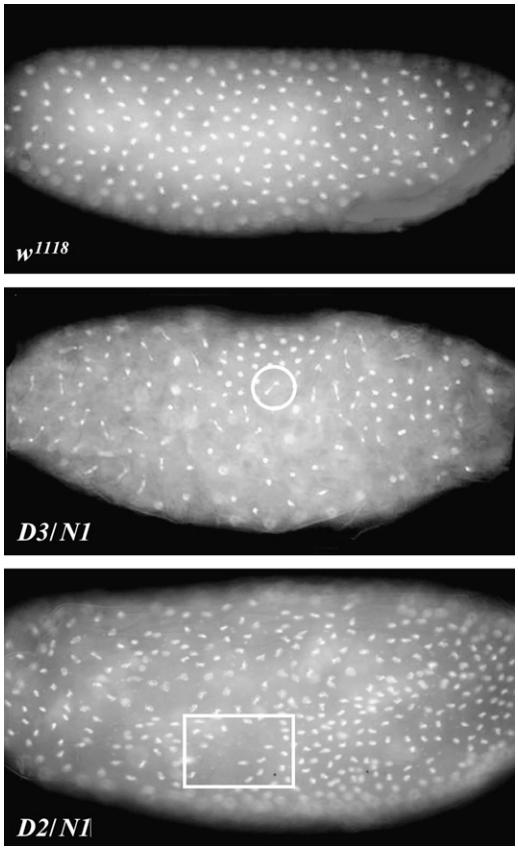

Figure 2.—Phenotypes of embryos from *mus309* mutant females. Representative DAPI-stained syncytial-stage embryos from wild-type (w^{1118}) or *mus309* mutant females are shown. Defects observed frequently include anaphase bridges (circle), gaps in the normally uniform monolayer of nuclei (box), and asynchronous mitoses (middle).

domain (*mus309*^{N2}, *mus309*^{N3}, and *mus309*^{N4}) were reduced relative to the hatch rate of embryos from wild-type females, but were much higher than for embryos from females carrying null alleles (Table 1). These embryos exhibited phenotypes similar to those described above, but the defects were less severe and less frequent.

To quantify the differences between embryos from the different maternal genotypes and to determine whether the defects that we observed are associated with failure to hatch, we examined other hallmarks of embryonic development. Nearly all embryos from wild-type mothers fixed 4–6 hr after egg laying cellularize and undergo gastrulation (Table 1). In contrast, fewer than half of the embryos from *mus309*^{N1}/*mus309*^{D2} females had cellularized by this time, and none had gastrulated, suggesting that development either was delayed or had ceased by this time. The discrepancy between the complete lack of gastrulation seen in this assay and the hatch rate of 4% (Table 1) may be due to a delay in development or the comparatively low number of embryos scored in this assay (100 total). Rates of cellularization and gastrulation among embryos from

TABLE 2

Meiotic nondisjunction in *mus309*^{N2} females

Genotype	B$^+$ ♀♀	B ♂♂	B ♀♀	B$^+$ ♂♂	*X* ndj (%)
Wild type	696	625	1	2	0.45
mus309^{N2}	1009	936	11	13	2.41
mus309^{N2}/*mus309*^{D2}	2520	1376	13	34	2.36

See MATERIALS AND METHODS for details of the crosses and derivation of progeny classes.

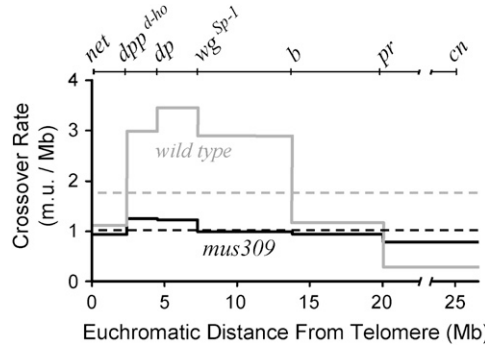

FIGURE 3.—Meiotic crossing over in *mus309*^{N2} females. Rates of meiotic crossing over in six intervals spanning distal *2L* to proximal *2R* are shown. The loci used in mapping are above the graph, placed along the *x*-axis according to physical distance along the chromosome. The gap between *pr* and *cn* indicates the position of the centromere and the pericentric heterochromatin, which is not included in the physical distances shown here. Solid lines show the number of map units (m.u.) per megabase pair (Mb) in each interval for wild-type females (shading) and *mus309*^{N2} females (solid). Dashed lines indicate the mean crossover rate across the entire region assayed for each genotype.

females homozygous for *mus309*^{N2} were much higher but were still reduced relative to those from wild-type females ($P < 0.0001$ for each comparison).

Our observations indicate that the sterility of *mus309* mutant females is due to a requirement for DmBlm in early embryogenesis. This appears to be a strict maternal effect, since zygotic mutants are fully viable (M. MCVEY and J. SEKELSKY, unpublished data). The intermediate severity observed when the N terminus is deleted may indicate that this region is dispensable for at least a subset of early embryonic functions of DmBlm. It is also possible that the RecQ helicase domain of DmBlm is sufficient for the essential embryonic function of DmBlm and that the intermediate phenotype of the N-terminal truncation alleles results from lower levels of the protein due to reduced expression or stability.

Meiotic recombination in *mus309* mutants: The findings presented above demonstrate that DmBlm has an essential function in embryogenesis, but do not rule out a role during meiosis. Previously, it has not been possible to assay meiotic recombination and chromosome segregation in *mus309* mutants, due to the extremely low hatch rates of embryos produced by *mus309*^{D2} and *mus309*^{D3} females. Females homozygous for *mus309*^{N2} produce a significant number of viable progeny, so we carried out these assays with this genotype. We first measured rates of nondisjunction of the *X* chromosome by mating *mus309*^{N2} females to males whose *Y* chromosome carried the dominant marker *B*^{S} (see MATERIALS AND METHODS). We measured a nondisjunction frequency of 2.4%, which is a 5-fold increase over the rate in wild-type females (Table 2; for unknown reasons, nondisjunction is 5- to 10-fold higher in our wild-type control than in typical controls, so the real increase in *mus309*^{N2} females may be higher). We measured a similar frequency of nondisjunction (2.3%) in *mus309*^{N2}/*mus309*^{D2} females.

Nondisjunction can result from defects in meiotic recombination (reviewed in HAWLEY 1988). To determine whether DmBlm plays a role in meiotic recombination, we generated wild-type and *mus309*^{N2} females that were heterozygous for multiple markers spanning the region from *net*, at the distal end of *2L*, to *cn*, on centromere-proximal *2R* (Figure 3). These females were testcrossed and the map distance between each marker was calculated (supplemental Tables S1, S2, and S3 at http://www.genetics.org/supplemental/). In *mus309*^{N2} females, the total map distance from *net* to *cn* was reduced by 46% relative to the distance in wild-type females (Figure 3). This decrease is consistent with the frequency of *X* nondisjunction that we measured (BAKER and HALL 1976).

The change in crossover frequency in *mus309*^{N2} females varied dramatically between different intervals. For example, in the two central intervals (*dp* to *wg*$^{Sp-1}$ and *wg*$^{Sp-1}$ to *b*), there was a 66% decrease in crossovers relative to wild-type females, but in the interval spanning the pericentric heterochromatin (*pr* to *cn*), crossover frequency was increased by 2.5-fold. The overall effect of these differences is that the crossover distribution in *mus309*^{N2} females is much more proportional to physical distance than the wild-type distribution (Figure 3). Thus, it appears that *mus309* belongs to the class that BAKER and CARPENTER (1972) referred to as "pre-condition mutants," meaning that they act prior to the time when crossovers are actually generated. A complication in interpreting the flattening of crossover distribution is that the data may include both meiotic crossovers and premeiotic germline crossovers (see DISCUSSION).

It is possible that defects in processing DSBs during meiotic recombination contribute to the embryonic phenotypes described above. To test this hypothesis, we generated *mei-P22*^{103} *mus309*^{N1} double mutants. MEI-P22 is required to generate meiotic DSBs (LIU *et al.* 2002), so meiotic recombination is not initiated in *mei-P22* mutants. Embryos from *mei-P22*^{103} *mus309*^{N1} double mutants exhibit phenotypes similar to those from *mus309*^{N1}

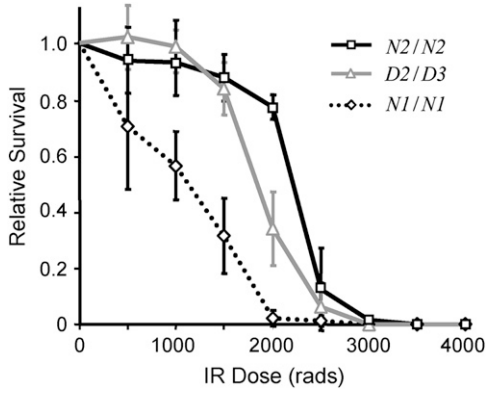

FIGURE 4.—Hypersensitivity of *mus309* mutants to ionizing radiation. Survival to adulthood of homozygous or heteroallelic mutants, relative to survival of heterozygous controls, is shown for three mutant genotypes for doses of gamma radiation up to 4000 rad. These doses do not have a large effect on survival of wild-type or heterozygous larvae (data not shown). Error bars indicate standard deviation from three independent experiments.

single mutants (data not shown), arguing that these phenotypes are not a consequence of defects in meiotic recombination.

The N terminus and the helicase domain of DmBlm are both required for repair of double-stranded gaps by SDSA: We have shown that embryonic defects are less severe for *mus309* alleles that do not affect the helicase domain than for those predicted to eliminate helicase activity. One possible explanation is that N-terminal truncations reduce protein stability and that the defects that we observed are actually due to a reduction in the amount of DmBlm helicase. Alternatively, or in addition, deletions predicted to cause N-terminal truncations may be separation-of-function alleles. To distinguish between these possibilities, we examined additional phenotypes.

A previous report showed that $mus309^{D2}/mus309^{D3}$ heteroallelic animals are hypersensitive to ionizing radiation (KOOISTRA *et al.* 1999). We tested various *mus309* allelic combinations to determine whether the different alleles have differences in IR sensitivity. In all cases, *mus309* homozygous or heteroallelic mutants were more sensitive than wild-type flies (Figure 4 and data not shown). At an intermediate dose of 2000 rad, mutants carrying alleles predicted to lack helicase activity ($mus309^{N1}$, $mus309^{D2}$) were more sensitive than those carrying the N-terminal deletion alleles $mus309^{N2}$ or $mus309^{N3}$. Interestingly, $mus309^{D2}/mus309^{D3}$ mutants exhibited less sensitivity than $mus309^{N1}/mus309^{N1}$ or $mus309^{N1}/mus309^{D2}$ mutants. These results suggest that the putative helicase-dead allele $mus309^{D3}$ and the N-terminal truncation alleles $mus309^{N2}$ and $mus309^{N3}$ retain some function that contributes to resistance to IR.

One cause of hypersensitivity to IR is defective DSB repair, and *mus309* mutants do have defects in repairing site-specific DSBs (BEALL and RIO 1996; ADAMS *et al.* 2003; MCVEY *et al.* 2004b; MIN *et al.* 2004; JOHNSON-SCHLITZ and ENGELS 2006b). To quantify the differences among different alleles, we used a *P*-element excision assay that can distinguish among different repair outcomes (ADAMS *et al.* 2003; MCVEY *et al.* 2004b). The $P\{w^a\}$ transgene carries a w^a allele that has a *copia* retrotransposon inserted into an intron of *w* (Figure 5), resulting in an apricot eye color in homozygous females or hemizygous males (females with one copy of $P\{w^a\}$ have yellow eyes), instead of a wild-type red eye color. Transposase-induced excision of the $P\{w^a\}$ element generates a 14-kb double-stranded gap, relative to the sister chromatid (the only template for repair, since excision occurs on the male *X* chromosome). Repair events that occurred in male premeiotic germline cells are recovered in daughters, in *trans* to an intact copy of $P\{w^a\}$.

Gap repair in Drosophila is best described by a modified version of the SDSA model (supplemental Figure S1 at http://www.genetics.org/supplemental/). For the *X* chromosome $P\{w^a\}$ element, repair almost always involves synthesis from both ends of the break, using the sister chromatid as a template (KURKULOS *et al.* 1994; ADAMS *et al.* 2003). It is thought that synthesis is not highly processive and that the nascent strand dissociates from the template after a few hundred nucleotides of synthesis (MCVEY *et al.* 2004a). Through repeated cycles of strand invasion, synthesis, and dissociation, the entire 14-kb gap can be filled, such that the ends of the nascent strands are complementary. Annealing of these complementary ends allows completion of repair by SDSA, resulting in restoration of the entire $P\{w^a\}$ element. Progeny that inherit this type of repair event have apricot eyes, but these cannot be distinguished from the 80–90% of progeny derived from cells in which $P\{w^a\}$ never excised. However, SDSA can also be completed when LTR sequences at the ends of the *copia* insertion anneal to one another. This generates a $P\{w^a\}$ derivative in which *copia* is deleted and a single LTR remains, allowing for nearly wild-type expression of the *w* gene and giving rise to progeny with red eyes. We use the number of red-eyed female progeny to estimate the frequency of completed SDSA.

Repair that results in loss of *w* expression can also occur, leading to female progeny with yellow eyes. In wild-type flies, the majority of these cases involve repair synthesis, usually from both ends of the break, followed by end joining. This type of repair, which we refer to as synthesis and end joining (S+EJ) yields a product that lacks parts of the *w* gene, rendering it nonfunctional. In some cases, repair products recovered in yellow-eyed progeny have deletions in genomic sequences flanking the $P\{w^a\}$ insertion site. For simplicity, we refer to all of this class of repair events as S+EJ.

To estimate the frequency of completed SDSA, we compared the percentage of progeny with red eyes (SDSA with annealing of LTR sequences) to the total percentage of progeny with any distinguishable repair

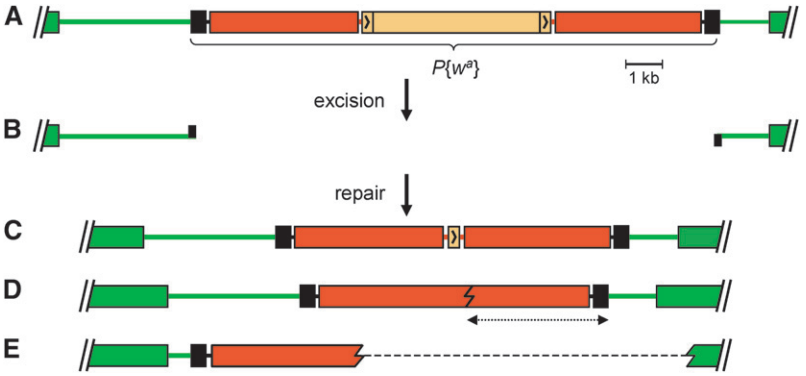

FIGURE 5.—Gap repair assay using $P\{w^a\}$. (A) Schematic of $P\{w^a\}$ structure. Green boxes represent exons of the *sd* gene. $P\{w^a\}$ is inserted into an intron of this gene. Black rectangles are *P*-element ends and red rectangles are the *w* gene. The *copia* retrotransposon (orange; LTRs indicated by carets) is inserted into an intron of *w*, decreasing expression such that homozygous females and hemizygous males have apricot-colored eyes, and hemizygous females have yellow eyes (MULLER 1932). (B) Transposase-induced excision of $P\{w^a\}$ leaves a break that has 17-nt 3′ overhangs of *P*-element sequence. Most repair is believed to occur through SDSA (KURKULOS *et al.* 1994; ADAMS *et al.* 2003; MCVEY *et al.* 2004a,b). Completed SDSA can restore the entire $P\{w^a\}$ (not shown). (C) Completion of SDSA can also involve annealing of the *copia* LTRs, producing a $P\{w^a\}$ derivative that retains only one LTR; daughters that inherit this repair product have red eyes. (D and E) The major classes of inaccurate repair. In both cases, the *w* gene becomes nonfunctional, so daughters that inherit either of these chromosomes will have yellow eyes due to the single copy of $P\{w^a\}$ inherited from the mother. In D, repair is initiated by SDSA, but is completed by end joining rather than annealing of complementary sequences. In most such cases, synthesis occurs from both ends of the break, as shown here. The extent of synthesis from the right end (dotted double-headed arrow) can be estimated through molecular analysis. (E) In some cases of inaccurate repair, products have a deletion in sequences adjacent to the $P\{w^a\}$ insertion site. In this example, there has been synthesis from the left end of the break and deletion to the right side (dotted line). Deletions can also be bidirectional. When a deletion extends near or into an exon of *sd*, as depicted here, the result is a male-lethal allele of *sd*. Deletions are uncommon in wild-type males, but frequent in *mus309* mutants.

event (red eyes or yellow eyes). This calculation corrects for differences in excision rate in different genetic backgrounds (see MATERIALS AND METHODS). SDSA was severely compromised in every *mus309* heteroallelic or homozygous combination tested (Figure 6 and supplemental Table S4 at http://www.genetics.org/supplemental/). The SDSA defect in mutants carrying the N-terminal truncation allele $mus309^{N2}$ was not significantly different from that of null mutants ($mus309^{N1}/mus309^{D2}$) in this assay (supplemental Table S5), in contrast to the milder phenotype with regard to embryonic development (Table 1) and IR sensitivity (Figure 4). Mutants with the putative helicase-dead allele $mus309^{D3}$ had a more severe phenotype than that of null mutants ($P < 0.001$ for each comparison; supplemental Table S5), even though these mutants appear to be hypomorphic in the IR sensitivity assay. To determine whether $mus309^{D3}$ is antimorphic in the gap repair assay, we tested $mus309^{D3}/+$ heterozygotes. Heterozygosity for the null allele $mus309^{D2}$ had no effect on SDSA ($P = 0.493$). In contrast, SDSA was significantly reduced in $mus309^{D3}/+$ heterozygotes ($P < 0.0001$ compared to wild type or $mus309^{D2}/+$), but not as severely as for any heteroallelic or homozygous mutant combination. Thus, the $mus309^{D3}$ allele appears to be semidominant for repair defects in this assay, consistent with it being antimorphic with respect to gap repair function; however, we cannot exclude the possibility that the $mus309^{D3}$ chromosome carries another mutation that increases the severity of this phenotype.

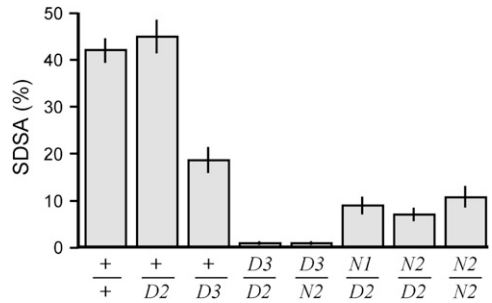

FIGURE 6.—Gap repair in *mus309* mutants. Bars indicate the fraction of repair products that occurred through SDSA with annealing of LTRs (see Figure 5C and MATERIALS AND METHODS). For each genotype, the maternally inherited allele is listed above the paternally inherited allele. Bars represent means and lines are standard errors of the mean. Number of vials, progeny, and red and yellow classes are given in supplemental Table S4 at http://www.genetics.org/supplemental/. For statistical analysis, see supplemental Table S5.

We previously reported that the decreased SDSA in $mus309^{D2}/mus309^{D3}$ flies is compensated by increased S+EJ and that these repair events had decreased synthesis tract lengths and an increased frequency of deletion into sequences adjacent to the DSB site (ADAMS *et al.* 2003; MCVEY *et al.* 2004b). These features were also seen in the heteroallelic and homozygous mutant genotypes that we tested here, although $mus309^{D3}/+$ females had no detectable decrease in synthesis tract length or increase in deletion frequency relative to wild type (supplemental Table S6 at http://www.genetics.org/supplemental/). On the basis of results from the *P*-element excision assay, we conclude that both the N terminus and the helicase domain of DmBlm are important for repair of double-strand gaps through SDSA and that loss of either part of DmBlm results in

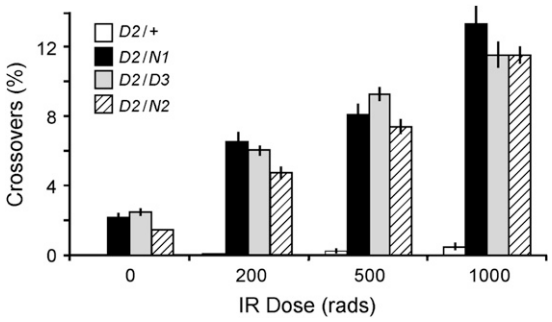

FIGURE 7.—Germline crossovers in wild-type and *mus309* mutant males. Bars show the mean percentage of progeny that were recombinant between *st* and *e*, with lines indicating standard error of the mean. Males either were untreated or were exposed to the indicated dose of gamma radiation during larval development. Crossover rates between different mutant genotypes were not significantly different, but at each dose each mutant genotype was significantly different from the wild type ($P < 0.0001$ for each comparison). See supplemental Table S7 at http://www.genetics.org/supplemental/ for numbers of vials, progeny, and crossovers.

decreased synthesis tract length and increased deletion into flanking sequences.

DmBlm prevents mitotic crossing over during DSB repair: A hallmark of cells from BS patients is increased mitotic crossing over between sister chromatids, homologous chromosomes, and heterologous chromosomes (GERMAN *et al.* 1977). We assayed the rate of mitotic crossing over between homologous chromosomes in *mus309* mutants. We measured crossover rates in the germlines of males because males do not have meiotic crossing over (MORGAN 1912), and, unlike *mus309* mutant females, *mus309* mutant males have normal fertility. We tested three heteroallelic combinations: $mus309^{N1}$, $mus309^{D3}$, and $mus309^{N2}$, each in *trans* to $mus309^{D2}$. The frequency of spontaneous germline crossing over between *scarlet* (*st*) on *3L* and *ebony* (*e*) on *3R* was significantly elevated for each of these genotypes relative to wild-type flies, but the mutant genotypes were not significantly different from one another (Figure 7 and supplemental Table S7 at http://www.genetics.org/supplemental/).

To determine whether defects in DSB repair in *mus309* mutants can lead to crossovers, we exposed larvae to various doses of ionizing radiation. IR induced a small number of crossovers in wild-type males, but even at 1000 rad the frequency was lower than the spontaneous crossover frequency in *mus309* mutant males (Figure 7 and supplemental Table S7 at http://www.genetics.org/supplemental/). Exposure to IR greatly increased the frequency of germline crossovers in *mus309* mutant males in a dose-dependent manner. At each dose, the frequency of crossovers in wild-type males was significantly lower than the frequency in mutant males, but the three different mutant genotypes were not significantly different from one another.

DISCUSSION

In this article, we describe several phenotypes associated with mutations that affect DmBlm. These phenotypes include defects in double-strand break repair (DSBR) in cycling cells, such as hypersensitivity to ionizing radiation, decreased ability to repair double-strand gaps by SDSA, and increased incidence of mitotic crossing over between homologous chromosomes. We observed defects in early embryogenesis, including frequent anaphase bridges, loss of syncytial nuclei, and developmental failure prior to gastrulation. Finally, we observed a reduced frequency of meiotic recombination and an altered crossover distribution. Analysis of the effects of different allelic combinations on some of these phenotypes provides insights into the relationships among the different functions for DmBlm. Each of these functions is discussed below.

DmBlm in embryogenesis: We have shown that the decreased fertility of *mus309* females is due to maternal-effect embryonic lethality. The vast majority of embryos obtained from mothers homozygous for null alleles of *mus309* displayed chromosome segregation defects prior to gastrulation (Table 1). Zygotically null mutants are fully viable (M. MCVEY and J. SEKELSKY, unpublished data), suggesting that the essential function for DmBlm is limited to early embryogenesis. This stage of development is characterized by rapid cycles of replication and nuclear division in a syncytium without intervening gap phases. These nuclei are able to achieve replication of the entire genome in 5–6 min at 25° by firing a large number of replication forks (KRIEGSTEIN and HOGNESS 1974; ZALOKAR and ERK 1976). We hypothesize that DmBlm facilitates resolution of converging replication forks during these rapid S phases, when other replication fork repair mechanisms may be unavailable. This is not unlike the roles proposed for Sgs1 and Rqh1 in decatenating converging replication forks in rDNA (FRICKE and BRILL 2003; COULON *et al.* 2004).

The defects in embryonic cell cycles were less severe in embryos derived from females homozygous for *mus309* alleles predicted to remove only the N terminus of DmBlm (Table 1). This alleviated phenotype may indicate that the N terminus is not essential for DmBlm embryonic function, in contrast to its requirement for repair of gaps by SDSA. It is also possible that the N-terminal truncation alleles have decreased maternal protein levels, either because an alternative start codon is used or because protein lacking the amino terminus has lower stability.

DmBlm in meiotic recombination: Null mutations in *mus309* result in almost complete female sterility (BOYD *et al.* 1981), but the fecundity of females homozygous for $mus309^{N2}$ is sufficient to allow studies of recombination that rely on scoring visible phenotypes in adult progeny. We measured crossing over in $mus309^{N2}$ mutant females

and found the frequency to be reduced to about half of the wild-type frequency (Figure 3). This result seems paradoxical, since loss of DmBlm results in increased mitotic crossing over in the male germline (Figure 7), consistent with the anticrossover activity associated with human BLM and yeast Sgs1 (Chaganti *et al.* 1974; Myung *et al.* 2001; Ira *et al.* 2003). Although modest increases in meiotic crossing over have been reported for *S. cerevisiae sgs1* mutants (Rockmill *et al.* 2003; Jessop *et al.* 2006), *Schizosaccharomyces pombe rqh1* mutants have a phenotype similar to what we observed: increased mitotic crossing over, but decreased meiotic crossing over (Ponticelli and Smith 1989; Stewart *et al.* 1997; Davis and Smith 2001).

The source of the decrease in meiotic crossing over in $mus309^{N2}$ females is unknown. It is possible that this decrease does not reflect a function for DmBlm in meiotic recombination *per se*, but may be a secondary consequence of some other meiotic function. For example, DmBlm may have a role in premeiotic DNA replication, and loss of this function may affect the ability of the oocyte to accomplish recombination. Another possibility is suggested by the finding that the anticrossover activity of *S. cerevisiae* Sgs1 is suppressed by structural components of meiotic chromosomes (Jessop *et al.* 2006). If DmBlm activity is regulated through physical interactions with meiotic chromosomes or the recombination machinery, then the absence of DmBlm could disrupt these structures, leading to an impairment of recombination.

The distribution of crossovers was also changed in $mus309^{N2}$ females. In Drosophila, as in most other eukaryotes, meiotic crossovers are distributed nonrandomly, such that there are lower-than-average rates in distal regions and near the pericentromeric heterochromatin than in the center of each chromosome arm. In $mus309^{N2}$ females, however, crossover distribution is roughly proportional to the physical euchromatic distance (Figure 3). There are a number of other mutations that decrease meiotic crossovers and alter the distribution of the residual crossovers (Baker and Carpenter 1972; Baker and Hall 1976; Carpenter and Sandler 1974; Carpenter and Baker 1982); however, the flattening of crossover distribution is more extreme in $mus309^{N2}$ females than in other mutants that have been analyzed. Interpretation of this result is complicated by our finding that this mutation increases premeiotic crossing over in the male germline. If this is also true for the female germline, then the crossovers that we recovered may include both mitotic and meiotic crossovers. Premeiotic germline crossovers are expected to appear in clusters corresponding to gametes derived from the cell in which the recombination event occurred. Consistent with this expectation, clusters of recombinants were often observed among the progeny of *mus309* mutant males (data not shown). It is more difficult to detect clustering of female germline events because of the physiology of oogenesis (*e.g.*, the cystoblast produced by a single stem-cell division yields 64 spermatids in the male germline, but only a single oocyte in the female germline) and because the high frequency of meiotic crossing over in females masks small clusters resulting from a single mitotic crossover. For these reasons, it is difficult to determine the extent to which the female crossover data that we recovered represents mitotic *vs.* meiotic crossovers. Nonetheless, it is likely that the flatness of the crossover distribution in $mus309^{N2}$ females (Figure 3) reflects a true randomization of the distribution of meiotic crossovers.

A further consideration in interpreting the female crossover data is that we do not know what effect the $mus309^{N2}$ allele has on meiotic recombination, relative to a null allele. In the DSB repair and male germline crossover assays (Figures 6 and 7), $mus309^{N2}$ behaves like a null, but with regard to IR sensitivity (Figure 4) and female fertility (Table 1), it is clearly hypomorphic. We attempted to address this by comparing nondisjunction in $mus309^{N2}$ homozygous females to nondisjunction in $mus309^{N2}/mus309^{D2}$ females (Table 2). The frequency of *X* nondisjunction was similar in both genotypes, but there was a qualitative difference: Similar numbers of sons and daughters were recovered from the $mus309^{N2}/mus309^{N2}$ mothers, but the ratios were significantly distorted among progeny of $mus309^{N2}/mus309^{D2}$ mothers. The cause of the sex-ratio bias is also unknown. Future experiments should provide insights into these phenotypes, as well as the other meiotic defects that we observed.

DmBlm in double-strand break repair: We report here that, like cells from BS patients, spontaneous crossing over is elevated in *mus309* mutants. Exposure to ionizing radiation causes a further increase in crossovers in the male germline. Ira *et al.* (2003) previously reported that *S. cerevisiae sgs1* mutants have elevated crossing over when site-specific DSBs are induced, and Johnson-Schlitz and Engels (2006b) recently reported a similar result in Drosophila *mus309* mutants. These results suggest that defective DSB repair is one source of the mitotic crossover elevation seen in the absence of BLM or orthologous proteins.

The dissolvase model (Figure 8A) has been proposed to explain the role of BLM in preventing crossovers (Ira *et al.* 2003; Wu and Hickson 2003). This proposal is based on the meiotic recombination model of Szostak *et al.* (1983), in which a structure with two Holliday junctions is a key intermediate in generating crossovers. Szostak *et al.* (1983) proposed that this double-Holliday junction (DHJ) structure is resolved by nicking two strands at each Holliday junction. Depending on which strands are nicked, resolution can produce crossover or noncrossover products. Thaler *et al.* (1987) suggested that resolution might also occur without nicking, if the two Holliday junctions are branch migrated toward one another and the remaining catenation is

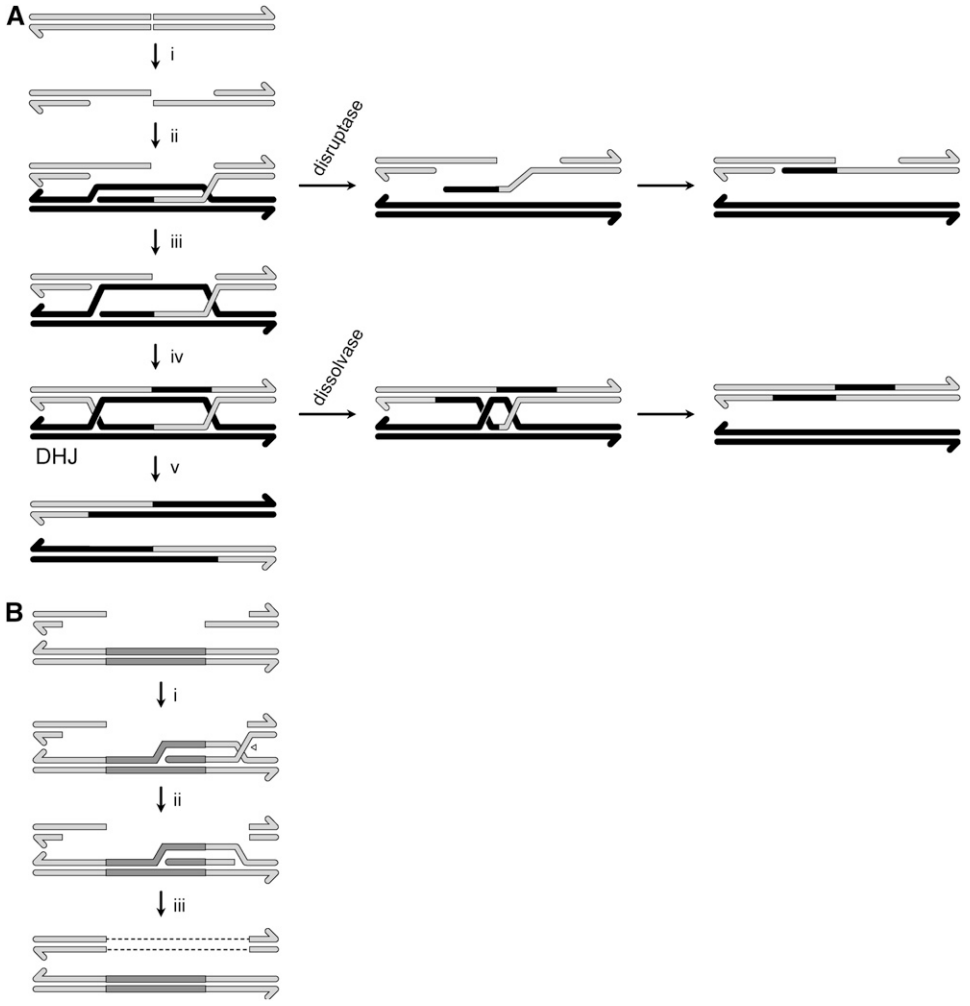

FIGURE 8.—Models for DmBlm function in DSB and gap repair. (A) Hypothesized functions for DmBlm in DSBR. (Left) The steps involved in generating a crossover, according to a modified version of the DSBR model of SZOSTAK *et al.* (1983). (i) Initial processing of the DSB involves resection of the 5′-ends, which generates 3′-ended, single-stranded overhangs. (ii) One overhang invades a homologous duplex, generating a D-loop, and the D-loop is enlarged when the invading strand is extended by repair DNA synthesis. (iii) The displaced strand anneals to the other 3′ overhang. (iv) Additional synthesis extends this end of the break, using the displaced strand as a template, and ligation at both ends leads to a DHJ. (v) The DHJ is resolved by nicking of two strands at each junction. In the example shown here, the two inner (crossing) strands are cut at the left junction, and the two outer (noncrossing) strands are cut at the right junction, giving rise to crossover chromatids. Resolution can also give rise to noncrossover chromatids. In the disruptase model, DmBlm removes the invading strand during or after synthesis, as in the SDSA model. The nascent sequence anneals to the other resected end, resulting a noncrossover repair product. In the dissolvase model, DmBlm migrates the two Holliday junctions toward one another. Decatenation by a type I topoisomerase generates a noncrossover repair product. (B) Hypothesis for the formation of deletions during gap repair. The first diagram illustrates a chromatid from which a transposable element (solid lines) has excised and the ends have been resected. (i) As in DSBR, a resected end invades a homologous template, such as the sister chromatid, and primes new synthesis. Displacement of the D-loop does not extend far enough to allow capture of the second end of the break. (ii) If DmBlm cannot dissociate the invading strand, this strand is cut (open arrowhead), resulting in an enlarged gap. (iii) If the two ends of the enlarged gap are repaired through end joining, as is common during gap repair in Drosophila, the product will lack the transposable element and will be deleted for sequences adjacent to the insertion site. If the left end of the break has invaded the sister chromatid and primed repair synthesis, it may contain some sequences from the left end of the transposable element or may also have a deletion. The fate of the template chromatid is not shown. Cutting of the invading strand may allow another helicase to remove the annealed strand, or it may be removed at the next S phase. A more complete illustration of this model for gap repair is given in supplemental Figure S1 at http://www.genetics.org/supplemental/.

removed by a topoisomerase; this process of "dissolution" generates only noncrossover products. In the dissolvase model, BLM is the Holliday junction branch migrating enzyme and topoisomerase 3α is the decatenating enzyme. Support for the dissolvase model comes from biochemical assays demonstrating that human BLM and TOP3α, as well as the Drosophila orthologs, can carry out this dissolution reaction *in vitro* (WU and HICKSON 2003; PLANK *et al.* 2006).

The dissolvase model does not easily explain the repair defects that we observe in *mus309* mutants (ADAMS *et al.* 2003; MCVEY *et al.* 2004b; this report). Gap repair in Drosophila is best explained by a modified version of the SDSA model (KURKULOS *et al.* 1994; NASSIF *et al.* 1994; ADAMS *et al.* 2003; MCVEY *et al.* 2004a). In this modified version (supplemental Figure S1 at http://www.genetics.org/supplemental/), a broken 3′-end invades a homologous duplex template, generating a D-loop, and primes repair synthesis. Synthesis is not highly processive, and the nascent strand is dissociated from the template after a few hundred nucleotides of synthesis. Experiments in *S. cerevisiae*, Drosophila, and

mammalian cells suggest that repair synthesis is not highly processive and that the nascent strand dissociates from the template frequently (Pâques *et al.* 1998; Richardson and Jasin 2000; McVey *et al.* 2004a; Smith *et al.* 2007). For a simple DSB, the newly synthesized strand can now anneal to the other end of the break, as in the canonical SDSA model. For a large gap, however, complementarity is generated only after repeated cycles of strand invasion, repair synthesis, and dissociation, which can take place from both ends simultaneously, since they are far apart. In some cases, multiple cycles of invasion, synthesis, and dissociation allow the entire gap to be filled. In our assay, this leads to restoration of the *P{w^a}* element. Alternatively, if synthesis from each end extends through the corresponding *copia* LTR and these anneal, the result is the repair product that we recover in red-eyed daughters. In many cases, the two ends are joined through a NHEJ pathway that is DNA ligase 4 independent (McVey *et al.* 2004c) before the gap is entirely filled. In our assay, this process of S+EJ results in partial restoration of the *P{w^a}* element (Figure 5D), which is the most common product among yellow-eyed daughters. Thus, the repair products that we detect in progeny with red eyes or yellow eyes are readily explained by a modified SDSA model, but are not easily explained by models that have a DHJ intermediate; it seems likely that the apricot-eyed progeny that result from excision and complete restoration also arise from this repair mechanism.

To explain the gap repair defects that we have described for *mus309* mutants, we proposed that DmBlm acts as a disruptase during SDSA (McVey *et al.* 2004b). In the disruptase model, DmBlm is the helicase that dissociates the invading and newly synthesized strand from the template (Figure 8A). This is similar to the activity proposed to explain the ability of *Escherichia coli* RecQ helicase to prevent illegitimate recombination by reversing unproductive strand invasions (Harmon and Kowalczykowski 1998), except that it occurs after repair synthesis. Support for a disruptase function comes from biochemical studies that show that BLM efficiently dissociates the invading strand from a D-loop substrate (van Brabant *et al.* 2000; Bachrati *et al.* 2006). Weinert and Rio (2007) recently demonstrated that DmBlm has both strand displacement and strand-annealing activity *in vitro*; they hypothesize that the combination of these activities promotes SDSA.

Although we proposed the disruptase model to explain the role of DmBlm in gap repair, this activity can also explain the anticrossover function of DmBlm. During repair of a DSB, rather than of a gap, inability to dissociate the invading strand might allow annealing of the strand displaced from the template to the other resected end of the break (Figure 8A). A DHJ intermediate could then be generated and resolved through nicking to produce crossover (or noncrossover) chromatids.

The disruptase model can also explain the finding that repair of the gap generated by excision of *P{w^a}* in *mus309* mutants is often accompanied by deletion into adjacent sequences (Adams *et al.* 2003; McVey *et al.* 2004b; supplemental Table S6 at http://www.genetics.org/supplemental/). We hypothesized that when the invading strand cannot be dissociated from the template by DmBlm, it is sometimes cleaved and that the ends of the break are then joined through NHEJ (Figure 8B). Johnson-Schlitz and Engels (2006b) recently reported the intriguing finding that deletions can occur on the template chromatid used for gap repair, a result that they interpreted as support for the dissolvase model. They suggested that DHJ intermediates that cannot be dissolved by DmBlm are cut so that both chromatids have DSBs and that these broken chromatids are repaired by NHEJ. If there is branch migration prior to Holliday junction cutting, the DSBs might be located far from the initial break site, yielding repair products with deletions. Depending on which ends are joined, a crossover may also result. This hypothesis presumes that DHJ intermediates are formed during repair of large gaps. As described above, we think this is unlikely. The gap generated in the experiments of Johnson-Schlitz and Engels (2006b) is only 5 kb, compared to the 14-kb gap used in our experiments (or 10-kb gap when LTRs anneal). Johnson-Schlitz and Engels (2006a) previously showed that a gap of 44 kb or larger is not repaired efficiently in Drosophila, whereas gaps of 11 kb or smaller are repaired. The 14-kb gap generated by excision of *P{w^a}* is within the range that is repaired efficiently (Adams *et al.* 2003; McVey *et al.* 2004b). Furthermore, we estimate that a typical repair synthesis event in Drosophila is on the order of a few hundred base pairs (McVey *et al.* 2004a), so, in both assays, completely filling the gap is likely to involve multiple cycles of strand invasion and synthesis.

It might still be possible to form a DHJ intermediate during gap repair if the entire single-stranded region, which can be thousands of nucleotides in length, undergoes strand invasion into a homologous duplex. If a DHJ intermediate is formed, dissolvase function of DmBlm may still prevent formation of crossovers. However, there is no reason to believe that failure to dissolve such a DHJ would lead to deletions. Several enzymes are known to resolve Holliday junctions, but these enzymes do so by nicking each duplex, not by generating DSBs (West and Korner 1985; Bennett and West 1995; Shah *et al.* 1997; Boddy *et al.* 2001; Constantinou *et al.* 2002). On the basis of these considerations, we believe that the dissolvase model does not easily explain the occurrence of deletions on the template chromatid. We speculate that these deletions could also result from loss of disruptase activity. We previously proposed that the invading strand of a D-loop is cleaved when it cannot be dissociated by DmBlm, but it is also possible that template strands are cut. This

would give the result proposed by Johnson-Schlitz and Engels (2006b): breaks on both chromatids, which can then by repaired by NHEJ. Indeed, it is possible that some of the events that we classified as deletions adjacent to the excision site are actually template deletions. Our experiments involve gap repair on the male *X* chromosome, so we cannot distinguish the excised chromatid from the sister chromatid that serves as a repair template.

Another argument that has been made in favor of the dissolvase model is that the absence of topoisomerase 3α results in a similar elevated mitotic crossover phenotype in *S. cerevisiae* and Drosophila (Ira *et al.* 2003; Johnson-Schlitz and Engels 2006b). In biochemical studies, dissolvase activity requires topoisomerase 3α (Wu and Hickson 2003; Plank *et al.* 2006), but disruptase activity does not (van Brabant *et al.* 2000; Bachrati *et al.* 2006; Weinert and Rio 2007). However, D-loop substrates used in *in vitro* studies are generated by annealing oligonucleotides, but D-loops generated *in vivo* occur in the context of chromosomes that are orders of magnitude longer than these model substrates. It is reasonable to expect that topoisomerases may be required for disruptase activity *in vivo*, where one or both ends of the template molecule are essentially immobilized.

The dissolvase and disruptase models are not mutually exclusive, and both may contribute to mechanisms through which BLM prevents crossing over or to other functions of BLM. We have argued that the disruptase function of DmBlm is more relevant during gap repair and perhaps during DSB repair in proliferating cells. Conversely, meiotic recombination events that occur in the absence of MEI-9, an endonuclease required to generate most meiotic crossovers in Drosophila, have the structure predicted by DHJ dissolution (Radford *et al.* 2007). We hypothesized that MEI-9 generates meiotic crossovers by cutting DHJ intermediates and that, in the absence of MEI-9, these DHJs undergo dissolution (Yildiz *et al.* 2004; Radford *et al.* 2007); DmBlm is a strong candidate for such a dissolvase.

It might be possible to distinguish between the disruptase and dissolvase hypotheses with separation-of-function mutations in *mus309*. We note that the gap repair defects and elevated rate of spontaneous mitotic crossovers are as severe in *mus309*^{N2} mutants as in null mutants. Human BLM lacking the region N-terminal to the helicase domain is proficient in carrying out the dissolution reaction *in vitro* (Wu *et al.* 2005). If, like human BLM, N-terminally deleted DmBlm is capable of carrying out dissolution, then the gap repair defects and elevated spontaneous crossovers that we observed must not result from loss of dissolvase activity. Conversely, the embryonic function for DmBlm may require only the dissolvase function or another function similar to Holliday junction branch migration. In the accompanying study of synthetic lethality between mutations in *mus81* and mutations in *mus309* (Trowbridge *et al.* 2007), we argue that the viability of *mus81; mus309*^{N2} mutants suggests that the *mus309*^{N2} mutation destroys the disruptase activity of DmBlm, but does not eliminate the ability to catalyze branch migration of Holliday junctions. The results reported therein, together with the findings described above, are consistent with the hypothesis that the disruptase activity of DmBlm is critical for DSB repair.

In conclusion, we have illustrated multiple functions for DmBlm in genome maintenance. Our results demonstrate that DmBlm is important for normal meiotic recombination, meiotic chromosome transmission, and embryonic development. We have also shown that DmBlm maintains genomic integrity in proliferating cells by inhibiting crossing over and by promoting accurate repair of double-stranded gaps. These findings highlight the multifunctional nature of DmBlm in the prevention of genomic instability.

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