

3. E. F. Eppens, N. Nouwen, J. Tommassen, *EMBO J.* **16**, 4295 (1997).
4. H. de Cock, J. Tommassen, *EMBO J.* **15**, 5567 (1996).
5. S. W. Lazar, R. Kolter, *J. Bacteriol.* **178**, 1770 (1996).
6. R. Chen, U. Henning, *Mol. Microbiol.* **19**, 1287 (1996).
7. S. Reumann, J. Davila-Aponte, K. Keegstra, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 784 (1999).
8. Materials and methods are available as supporting material on Science Online.
9. K. L. Thomas *et al.*, *Infect. Immun.* **69**, 4438 (2001).
10. C. Jansen *et al.*, *Biochim. Biophys. Acta* **1464**, 284 (2000).
11. M. Wolfgang, J. P. van Putten, S. F. Hayes, D. Dorward, M. Koomey, *EMBO J.* **19**, 6408 (2000).
12. T. Prinz, J. Tommassen, *FEMS Microbiol. Lett.* **183**, 49 (2000).
13. N. Dekker, J. Tommassen, A. Lustig, J. P. Rosenbusch, H. M. Verheij, *J. Biol. Chem.* **272**, 3179 (1997).
14. R. G. Brok *et al.*, *J. Bacteriol.* **176**, 861 (1994).
15. J. Pohlner, R. Halter, K. Beyreuther, T. F. Meyer, *Nature* **325**, 458 (1987).
16. I. R. Henderson, F. Navarro-Garcia, J. P. Nataro, *Trends Microbiol.* **6**, 370 (1998).
17. D. Martin, N. Cadieux, J. Hamel, B. R. Brodeur, *J. Exp. Med.* **185**, 1173 (1997).
18. M. Fussenegger, A. F. Kahrs, D. Facius, T. F. Meyer, *Mol. Microbiol.* **19**, 1357 (1996).
19. L. Steeghs *et al.*, *Nature* **392**, 449 (1998).
20. L. Steeghs *et al.*, *EMBO J.* **20**, 6937 (2001).
21. B. Bolter, J. Soll, A. Schulz, S. Hinnah, R. Wagner, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15831 (1998).
22. D. S. Manning, D. K. Reschke, R. C. Judd, *Microb. Pathog.* **25**, 11 (1998).
23. S. Reumann, K. Keegstra, *Trends Plant. Sci.* **4**, 302 (1999).
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Drosophila BLM in Double-Strand Break Repair by Synthesis-Dependent Strand Annealing

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Bloom syndrome, characterized by a predisposition to cancer, is caused by mutation of the RecQ DNA helicase gene *BLM*. The precise function of BLM remains unclear. Previous research suggested that *Drosophila* BLM functions in the repair of DNA double-strand breaks. Most double-strand breaks in flies are repaired by homologous recombination through the synthesis-dependent strand-annealing pathway. Here, we demonstrate that *Drosophila* BLM mutants are severely impaired in their ability to carry out repair DNA synthesis during synthesis-dependent strand annealing. Consequently, repair in the mutants is completed by error-prone pathways that create large deletions. These results suggest a model in which BLM maintains genomic stability by promoting efficient repair DNA synthesis and thereby prevents double-strand break repair by less precise pathways.

Bloom syndrome (BS) is an autosomal recessive disorder characterized by short stature, immunodeficiency, sterility, and early development of a wide spectrum of cancers (1). BS cells exhibit extreme genomic instability (2). The gene mutated in BS, *BLM*, encodes a RecQ helicase (3) that preferentially unwinds or migrates recombination intermediates, including Holliday junctions and D loops (4). BLM interacts with homologous recombination proteins, including RAD51, RPA, and MLH1 (5–7). These findings suggest that BLM functions in homologous recombination. However, the hyper-recombination phenotype of BS cells argues that BLM prevents inappropriate recombination. Therefore, the precise biological function of BLM remains unclear.

The *Drosophila* ortholog of BLM (DmBlm) is encoded by the *mus309* gene (8). Mutations in *mus309* cause phenotypes similar to those of BS, including reduced fertility, increased non-

disjunction and chromosome loss, and elevated mitotic exchange (8–10). In addition, *mus309* mutants are sensitive to excision of P elements and other agents that cause double-strand breaks (DSBs) (10). Thus, DmBlm likely plays an important role in double-strand break repair (DSBR).

DSBR in *Drosophila* usually occurs by homologous recombination (11), primarily through the synthesis-dependent strand-annealing (SDSA) pathway (12, 13). In the SDSA model (14), processing of a DSB generates 3' single-stranded tails, which invade homologous templates and prime DNA synthesis. Nascent DNA strands are displaced from the template and can then anneal with complementary DNA from the recipient chromosome. SDSA therefore results in gene conversion without crossing over, in contrast to homologous recombination pathways that involve resolution of Holliday junction intermediates to produce crossovers (15). Because a hallmark of BS cells is increased crossing over (e.g., sister chromatid exchange), BS cells may be defective in a primary DSBR pathway that does not generate crossovers, such as SDSA.

We employed a P-element system to address whether DmBlm is involved in SDSA. *P{w^a}* is a P element that carries the *apricot* allele of the

white (w) gene (Fig. 1) (12). The *w⁺* gene confers red eye color. In the *white-apricot (w^a)* allele, a *copia* insertion in an intron decreases expression of the *white* gene, so that flies with one copy of *w^a* have yellow eyes and those with two copies have apricot-colored eyes.

P transposase catalyzes excision of *P{w^a}* by making a DSB at each end of the element (16). We used a copy of *P{w^a}* inserted into an intron of the essential X-linked *scalloped (sd)* gene and induced excision in males, in which the only template for homologous repair is the sister chromatid. The *copia* retrotransposon in *w^a* has directly repeated 276-base pair (bp) long terminal repeats (LTRs) at each end. When a DSB created by excision of *P{w^a}* is repaired by SDSA, synthesis from each end of the break generates regions of single-stranded DNA that are complementary at the LTRs. If nascent LTR sequences anneal to one another during repair, the product is *P{w^{aLTR}}*, containing only a single LTR in the *white* intron. This allows for near wild-type expression of *white*, generating a flat red eye color (Fig. 1). The appearance of red eyes therefore directly measures repair by SDSA (12).

We observed that wild-type males carrying *P{w^a}* and P transposase had mosaic eyes, owing to excision and repair in mitotic cells in the developing eye (fig. S1) (17). Frequent repair by SDSA was apparent from the presence of patches of red cells. These males also had repair events in developing mitotic germ line cells, which we sampled by crossing the males to females homozygous for *P{w^a}*. Eye color in the female progeny allowed us to classify repair events into three categories (Fig. 1).

Ninety-two percent of the progeny had apricot-colored eyes. Most of these probably derived from cells in which *P{w^a}* did not excise, but some may have resulted from excision followed by repair to restore the complete *P{w^a}* element (e.g., SDSA without annealing between LTRs). Almost 4% of the progeny had flat red eyes, as found in a previous study (12). We confirmed that >90% of the red-eyed flies ($n = 70$) had a single LTR remaining at the *copia* insertion site. Therefore, these progeny arose from SDSA with annealing between LTRs.

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About 4% of the progeny had yellow eyes, corresponding to aberrant repair events that resulted in the loss of *white* activity (the yellow color is from $P\{w^a\}$ inherited from the mother).

To determine whether the loss of DmBlm affects SDSA, we repeated this assay in *mus309* mutant males. Mutants carrying $P\{w^a\}$ and P transposase were nearly devoid of red eye patches, suggesting a defect in SDSA (fig. S1). Among the progeny of these males, only 0.1% had flat red eyes, a decrease by a factor of 38 from the percentage of wild-type flies with flat red eyes from wild-type males (Fig. 1). In addition, there were fewer apricot-eyed progeny, as compared with the number from wild-type flies, which we attribute to an inability to restore the entire $P\{w^a\}$ element by SDSA.

The decreased number of apricot- and red-eyed progeny from *mus309* mutants shows that successful SDSA rarely occurs in the absence of DmBlm. Conversely, the frequency of yellow-eyed females, representing aberrant repair events, was increased 3.5-fold among the progeny *mus309* mutants. This indicates that DSB in the absence of DmBlm occurs through error-prone pathways that result in a complete loss of *white* activity. The normal function of DmBlm may be to prevent DSB through these error-prone pathways. Alternatively, DmBlm may play a direct role in SDSA. To distinguish between these possibilities, we examined the aberrant repair events that gave rise to yellow-eyed progeny.

We first asked whether SDSA was initiated in DmBlm mutants by assaying DNA synthesis from each end of the DSB (Fig. 2A). Most aberrant repair events from wild-type males (96%) had evidence of repair synthesis on one or both sides of the DSB. Similarly, most of the aberrant events that occurred in *mus309* mutants (88%) exhibited repair synthesis from at least one end. Although evidence for repair synthesis was less frequent in *mus309* mutants, the decrease does not account for the decrease by a factor of 38 in red-eyed progeny. We suggest that in the absence of DmBlm, DSBs enter the SDSA pathway, but these repair attempts fail after the initiation of repair synthesis.

We then compared synthesis tract lengths in aberrant repair events. Analysis of the right side revealed that 80% of these events generated in wild-type males involved at least 920 bp of repair synthesis and that 20% had at least 4674 bp of synthesis (Fig. 2B). Repair events represented by red-eyed flies had to involve at least 4600 bp of synthesis to reach the *copia* LTR. We conclude that repair DNA synthesis in wild-type males usually involves several kilobases. In contrast, aberrant repair events generated in the absence of DmBlm had shorter synthesis tracts: Only 21% had at least 920 bp of synthesis at the right end, and none synthesized as far as 4674 bp. Thus, *mus309* mutants are severely impaired

in their ability to carry out extensive repair DNA synthesis.

After processing of a DSB, exonucleolytic degradation of the ends may compete with extension by repair DNA synthesis (18). A mutation that reduces repair synthesis may therefore result in larger or more frequent deletions of sequences flanking the DSB. In our assay, flanking deletions can result in lethal *sd* alleles. Among 71 aberrant repair events generated in wild-type males, only 1 had a lethal *sd* mutation; in contrast, 40 of 147 aberrant repair events generated in the absence of DmBlm had lethal *sd* mutations. Hence, loss of DmBlm frequently results in deletions into flanking sequences.

The shorter synthesis tracts in *mus309* mutants suggest a direct function for DmBlm in repair synthesis during SDSA. Alternatively,

DmBlm may prevent premature termination of repair synthesis. Biochemical studies indicate that *Escherichia coli* RecQ prevents illegitimate recombination by unwinding annealing at microhomologies (19). If DmBlm functions similarly, we would expect to see frequent joining at microhomologies in *mus309* mutants. To test this hypothesis, we sequenced aberrant repair junctions (Table 1, table S2). In one class, about half of the junctions contained 1- to 10-bp microhomologies (class 1). A second class had no apparent homology at the junction. In the third class, some repair events involved the insertion of nucleotides before joining. The ratios of these three classes were similar in wild-type flies and *mus309* mutants. Thus, the mechanisms of aberrant repair are independent of DmBlm, suggesting that DmBlm promotes repair synthesis.

DmBlm may promote repair synthesis di-

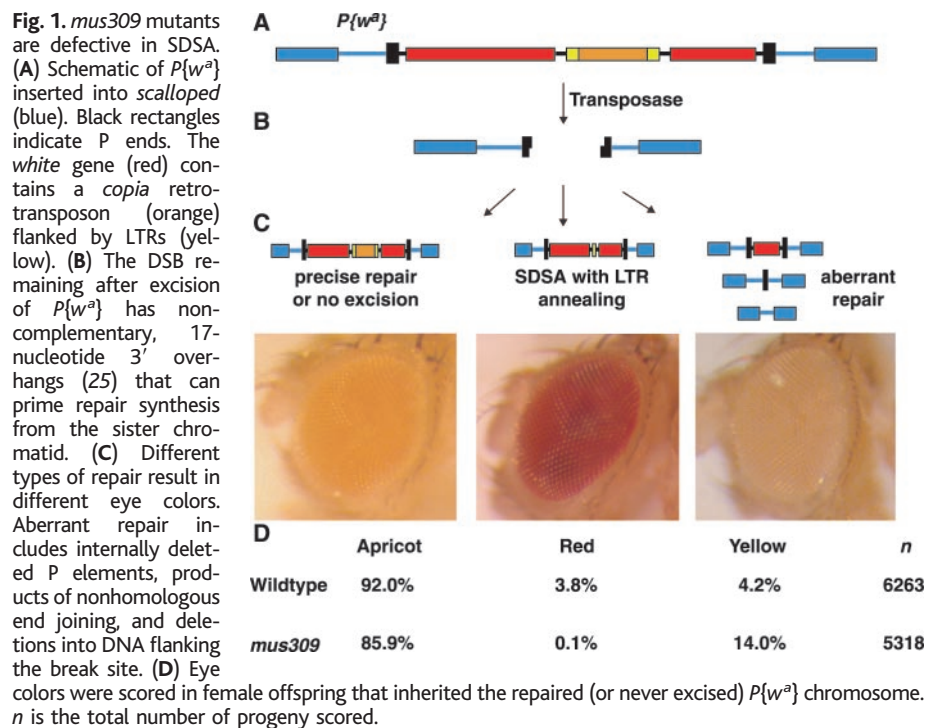
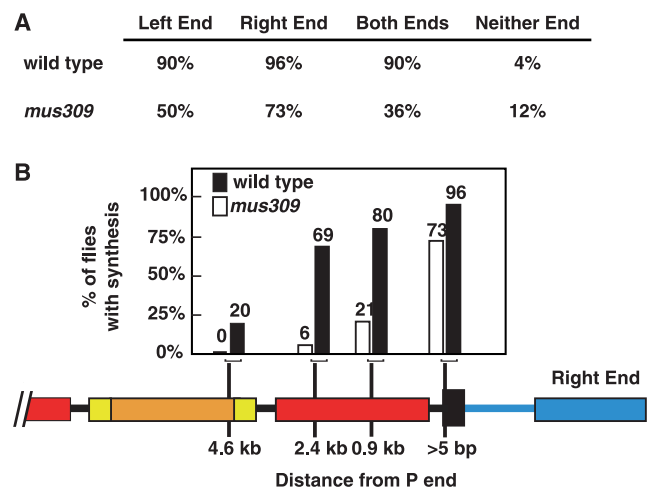


Fig. 2. Repair synthesis is reduced in *mus309* mutants. (A) The ends of the DSB site were analyzed by polymerase chain reaction to detect repair synthesis (17). The percentage of yellow-eyed females with evidence of repair synthesis from each P end is given. (B) Repair synthesis from the right end of the DSB was analyzed for 71 independent aberrant events from wild-type flies and 147 aberrant events from *mus309* mutants. The percentages that exhibited repair synthesis tracts of at least the lengths indicated are shown.



rectly or indirectly. DmBlm could act ahead of the replication fork to unwind the template, perhaps at regions that are difficult for the repair machinery to traverse. It is also possible that DmBlm acts before repair synthesis; for example, DmBlm may facilitate strand invasion through an interaction with Rad51.

If repair synthesis is not processive, repeated rounds of strand invasion may be necessary to replicate across large gaps, and a decrease in the efficiency of strand invasion could result in shorter synthesis tracts.

Richardson and Jasin (20) proposed that mammalian cells couple conventional nonho-

mologous end joining with homologous recombination when homology is constrained. Most junctions that we sequenced had properties consistent with repair by end joining after aborted repair synthesis (Fig. 3). However, some junctions that we examined showed de novo addition of short DNA sequences. Inspection of these sequences usually revealed a possible template for the insertion near the junction. Such templated nucleotides (T nucleotides) have been reported in *Drosophila* P-element excision repair (21, 22) and, more recently, in chromosome translocations associated with follicular and mantle cell lymphomas (23, 24). We suggest that the presence of T nucleotides is characteristic of aberrant end joining after aborted homologous recombination.

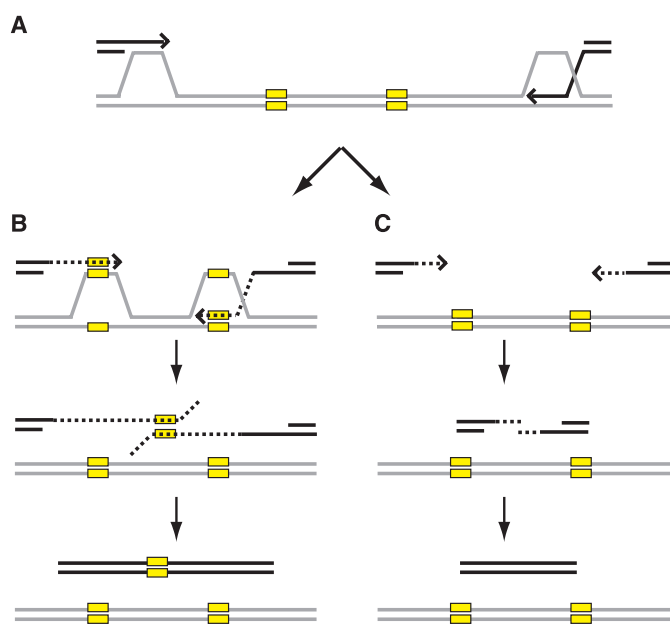
DSB repair by SDSA preserves genomic integrity. The results reported here demonstrate that DmBlm is required for SDSA. In the absence of DmBlm, cells are forced to repair DSBs by pathways that frequently generate large deletions. Likewise, human BS cells also may be unable to repair DSBs accurately, resulting in genomic instability and cancer predisposition.

Table 1. Junction sequences of aberrant repair products. Representative sequences of each class are given; a list of all sequenced junctions is available in table S2. WT indicates wild-type flies, and M indicates *mus309* mutants. The left and right boundaries are given relative to the ends of $P\{w^a\}$, with positive values reading into the P element and negative values indicating a loss of flanking sequences. The sequence surrounding each junction is given with left and right ends demarcated by colons. Uppercase letters between colons represent microhomologies, and lowercase letters represent sequence additions. T nucleotides and their putative templates are underlined.

Isolate	Boundaries (bp)	Sequence
<i>Class 1: junctions with microhomologies</i>		
WT1	5475/2532	GAGGCTGCTACTGAG:TTC:TCTAGCCACTCAGTG
WT2	2098/2685	CATT TGAGCGAACCG:AAT:T TAT T TTTCAAACG
WT3	1909/2572	CTTTCAGT TCAAAT:T:G:CTCACTGCTCATCT
M1	15/57	CCATGATGAAATAAC:A:GCATACGT TAAGTGG
M2	205/5	CTGGAGTAAAAT TAA:T TCA:TCATGACCCAGACTC
M3	-1289/112	ATACTAAACATAT TG:TCA:CTCAGACTCAATACG
<i>Class 2: junctions without microhomologies</i>		
WT8	5634/5631	ACCGCTACCGTCGAC:GAAT T TCCCTTGAAT
WT9	3905/5176	ATGTAATGCTAGATA:ATAAGTTCGCAAAA
WT10	1394/2594	GTTGCCACGT TGGAA:T TGCATT TCCCTCCT
M19	131/16	T T TGA AAACAT TAAC:ATG T TAT T T C ATCAT
M20	189/116	GCAAAGCTGTGACTG:TCAC T CAGACTCAAT
M21	400/1134	AGAGCCTGAACCAGA:TCTTGATCATGATAT
<i>Class 3: junctions with insertions</i>		
WT11	3191/5367	CAAATG TAT TCTAAA: <u>tgaaatga</u> *:CGT TGTGGTCATTTT
WT12	2561/287	CTCCAGGATGACCTT: <u>ctattctagg</u> :GGGATTCTAGGGGGA
WT13	12/258	GACCATGATGAAATA: <u>tc:GATCCGTCGACCTGC</u>
M26	53/141	AAGCT TACCGAAGTA: <u>atcaa</u> :GGT TAATCAACAATC
M27	66/-2069	TATACACT TAAAT TC: <u>tttt</u> :TAT TCT TTTT TTTT
M28	217/-893	TCACGTGCCGAAGTG: <u>ccgaag</u> :T TGA AAAACCAT TGC

*A possible template is located 67 bp to the left of the junction.

Fig. 3. Model for $P\{w^a\}$ repair in wild-type and *mus309* flies. (A) After excision of $P\{w^a\}$, the processed ends of the DSB (black) invade the homologous sister chromatid (gray) and prime repair synthesis (dotted lines). (B) As repair synthesis proceeds past the LTRs (yellow), annealing of complementary sequences can occur, resulting in the loss of an LTR and the intervening *copia* sequence. Alternatively, repair synthesis can proceed beyond the LTRs to restore the entire $P\{w^a\}$ element (not shown). (C) Occasionally, repair synthesis is initiated but does not proceed past the LTRs. These aborted SDSA events are completed by error-prone pathways. One such pathway, alignment at a microhomology, is shown here. Most of the repair events in *mus309* mutants occur through these error-prone pathways.



References and Notes

1. J. German, *Medicine* **72**, 393 (1993).
2. R. S. Chaganti, S. Schonberg, J. German, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4508 (1974).
3. N. A. Ellis et al., *Cell* **83**, 655 (1995).
4. J. K. Karow et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6504 (2000).
5. R. M. Brosh Jr. et al., *J. Biol. Chem.* **275**, 23500 (2000).
6. G. Pedrazzi et al., *Nucleic Acids Res.* **29**, 4378 (2001).
7. L. Wu et al., *J. Biol. Chem.* **276**, 19375 (2001).
8. K. Kusano et al., *Science* **291**, 2600 (2001).
9. J. B. Boyd et al., *Genetics* **97**, 607 (1981).
10. E. L. Beall, D. C. Rio, *Genes Dev.* **10**, 921 (1996).
11. W. R. Engels et al., *Cell* **62**, 515 (1990).
12. M. Kurkulos et al., *Genetics* **136**, 1001 (1994).
13. N. Nassif et al., *Mol. Cell. Biol.* **14**, 1613 (1994).
14. T. Formosa, B. M. Alberts, *Cell* **47**, 793 (1986).
15. F. Pâques, J. E. Haber, *Microbiol. Mol. Biol. Rev.* **63**, 349 (1999).
16. P. D. Kaufman, D. C. Rio, *Cell* **69**, 27 (1992).
17. Materials and methods are available as supporting material on Science Online.
18. G. B. Gloor et al., *Science* **253**, 1110 (1991).
19. F. G. Harmon, S. C. Kowalczykowski, *Genes Dev.* **12**, 1134 (1998).
20. C. Richardson, M. Jasin, *Mol. Cell. Biol.* **20**, 9068 (2000).
21. K. O'Hare, G. M. Rubin, *Cell* **34**, 25 (1983).
22. B. E. Stavely et al., *Genetics* **139**, 1321 (1995).
23. U. Jäger et al., *Blood* **95**, 3520 (2000).
24. N. Welzel et al., *Cancer Res.* **61**, 1629 (2001).
25. E. L. Beall, D. C. Rio, *Genes Dev.* **11**, 2137 (1997).
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