Drosophila FANCM Helicase Prevents Spontaneous Mitotic Crossovers Generated by the MUS81 and SLX1 Nucleases

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ABSTRACT Several helicases function during repair of double-strand breaks and handling of blocked or stalled replication forks to promote pathways that prevent formation of crossovers. Among these are the Bloom syndrome helicase BLM and the Fanconi anemia group M (FANCM) helicase. To better understand functions of these helicases, we compared phenotypes of *Drosophila melanogaster Blm* and *Fancm* mutants. As previously reported for BLM, FANCM has roles in responding to several types of DNA damage in preventing mitotic and meiotic crossovers and in promoting the synthesis-dependent strand annealing pathway for repair of a double-strand gap. In most assays, the phenotype of *Fancm* mutants is less severe than that of *Blm* mutants, and the phenotype of *Blm Fancm* double mutants is more severe than either single mutant, indicating both overlapping and unique functions. It is thought that mitotic crossovers arise when structure-selective nucleases cleave DNA intermediates that would normally be unwound or disassembled by these helicases. When BLM is absent, three nucleases believed to function as Holliday junction resolvases—MUS81-MMS4, MUS312-SLX1, and GEN—become essential. In contrast, no single resolvase is essential in mutants lacking FANCM, although simultaneous loss of GEN and either of the others is lethal in *Fancm* mutants. Since *Fancm* mutants can tolerate loss of a single resolvase, we were able to show that spontaneous mitotic crossovers that occur when FANCM is missing are dependent on MUS312 and either MUS81 or SLX1.

HELICASES are best known as enzymes that separate the strands of duplex nucleic acids, but many DNA repair helicases process more complex structures to direct repair pathways toward specific outcomes (reviewed in Brosh 2013). The Bloom syndrome helicase (BLM) has activities that promote disassembly of D loops and double-Holliday junction (dHJ) intermediates (Karow *et al.* 2000; Van Brabant *et al.* 2000; Wu and Hickson 2003). These activities prevent formation of crossovers during repair of DNA double-strand breaks (DSBs) (reviewed in Andersen and Sekelsky 2010). Disassembly of a D loop is a key step in the noncrossover synthesis-dependent strand annealing (SDSA) pathway. *Drosophila* BLM plays an important role in SDSA during gap repair, most likely by promoting D-loop disassembly after repair synthesis (Adams *et al.* 2003; McVey *et al.* 2004b). Likewise, the *Saccharomyces cerevisiae* ortholog Sgs1 generates noncrossovers during meiotic DSB repair and mitotic gap repair (De Muyt *et al.* 2012; Mitchel *et al.* 2013). If D loops are not disassembled, repair may proceed to generate a dHJ intermediate. BLM collaborates with topoisomerase 3α and other proteins to disassemble dHJs into noncrossover products *in vitro*, a process termed "dissolution" (Wu and Hickson 2003). Evidence for dHJ dissolution *in vivo* comes from meiotic return-to-growth experiments and mitotic gap repair assays in budding yeast (Dayani *et al.* 2011; Mitchel *et al.* 2013). If a dHJ is not dissolved, it must be resolved by structure-selective endonucleases (resolvases), which may generate reciprocal crossover products.

FANCM helicase also prevents crossing over (reviewed in Whitby 2010). *FANCM* mutations in humans cause Fanconi anemia (FA), a hereditary disorder characterized by developmental abnormalities, bone marrow failure, and cancer predisposition (reviewed in Soulier 2011). Cells from FA patients exhibit

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heightened sensitivity to agents that cause DNA interstrand crosslinks (ICLs), suggesting a defect in ICL repair (reviewed in Kim and D'Andrea 2012). FANCM is thought to function during an early step in the FA repair pathway, perhaps in damage recognition and recruitment of additional FA proteins. FANCM also has functions outside of the FA pathway. Anticrossover functions for FANCM and its orthologs have been observed in several contexts. Spontaneous sister-chromatid exchange is elevated in mouse embryonic fibroblasts and chicken DT40 cells that lack FANCM (Mosedale *et al.* 2005; Bakker *et al.* 2009). Orthologs in *S. cerevisiae* (Mph1) and *Schizosaccharomyces pombe* (Fml1) promote noncrossover outcomes during mitotic DSB repair (Sun *et al.* 2008; Prakash *et al.* 2009; Mazón and Symington 2013; Mitchel *et al.* 2013), and meiotic crossovers are elevated in *Arabidopsis FANCM* mutants and *S. pombe fml* mutants (Crismani *et al.* 2012; Lorenz *et al.* 2012). Like BLM, FANCM and its orthologs can branch-migrate HJs and disassemble D loops (Gari *et al.* 2008; Sun *et al.* 2008; Prakash *et al.* 2009). FANCM is not thought to not be capable of catalyzing dissolution; hence, it has been proposed that the D-loop disassembly activity of Fml1 and Mph1 promotes SDSA, thereby preventing formation of dHJs and resolution of these into crossovers (Sun *et al.* 2008; Prakash *et al.* 2009; Mitchel *et al.* 2013).

Biochemical and genetic studies have identified several likely nuclear HJ resolvases (reviewed in Schwartz and Heyer 2011). Among these, GEN1/Yen1 appears to have the greatest selectivity for HJs, but also has activity on 5′ flaps and replication fork-like structures (Ip *et al.* 2008). Genetic studies in vertebrate cells, budding yeast, and *Caenorhabditis elegans* have failed to identify a major function for GEN1 or its orthologs in generating meiotic or mitotic crossovers (Blanco *et al.* 2010; Ho *et al.* 2010; Tay and Wu 2010; Wechsler *et al.* 2011; Agostinho *et al.* 2013; Saito *et al.* 2013). In budding yeast, Yen1 functions are revealed in *mus81 yen1* double mutants, leading to the hypothesis that Yen1 functions as a backup to the Mus81–Mms4 endonuclease (Blanco *et al.* 2010; Ho *et al.* 2010; Tay and Wu 2010; Wechsler *et al.* 2011).

Mus81–Mms4/Eme1 and its orthologs have important roles in generating meiotic and mitotic crossovers in several organisms (Boddy *et al.* 2001; De Los Santos *et al.* 2003; Berchowitz *et al.* 2007; Ho *et al.* 2010; Wechsler *et al.* 2011). Although this enzyme was reported to cut HJs (Boddy *et al.* 2001; Chen *et al.* 2001), studies with recombinant protein found that intact HJs are not a good substrate, but nicked HJs, D loops, and 3′ flaps are (Ehmsen and Heyer 2008). This apparent paradox suggested models in which crossovers are generated by cleavage of a D loop (Whitby 2005) or a structure with nicked HJs (Osman *et al.* 2003; Schwartz and Heyer 2011). Another solution suggested by recent studies is that Mus81–Mms4/Eme1 functions as an HJ resolvase together with the Slx1 nuclease, with Slx1 making the first nick and Mus81 then cutting the nicked HJ (Castor *et al.* 2013; Garner *et al.* 2013; Wyatt *et al.* 2013). Human SLX1 was previously shown to have HJ resolution activity *in vitro*; this activity is dependent on SLX4, a scaffolding protein that also interacts with MUS81–EME1 (Fekairi *et al.* 2009; Muñoz *et al.* 2009; Svendsen *et al.* 2009). Thus, it is proposed that SLX4 coordinates the activities of SLX1 and MUS81–EME1 to coordinately resolve HJs.

Not surprisingly, helicase and resolvase gene mutations often show genetic interactions. In *S. cerevisiae*, *sgs1* mutations are synthetically lethal with mutations in *mus81* or *mms4* and with mutations in *slx1* or *slx4* (Kaliraman *et al.* 2001; Fricke and Brill 2003). Lethality of *sgs1 mus81* mutants is suppressed by mutations that prevent recombination, but lethality of *sgs1 slx1* (or *slx4*) is not, suggesting different causes for the lethality.

The above discussion hints at the inherent functional complexity between BLM, FANCM, and resolvases. We have sought to tease apart some of this complexity through genetic studies in the model metazoan *Drosophila melanogaster*. Mitotic crossovers are highly elevated in *Drosophila Blm* mutants, and these mutants have defects in SDSA and meiotic recombination (Adams *et al.* 2003; McVey *et al.* 2004a,b; Kohl *et al.* 2012). *Blm* mutations are synthetically lethal with mutations in *mus81*, *mus312* (encodes the ortholog of Slx4), *Slx1*, or *Gen* (Trowbridge *et al.* 2007; Andersen *et al.* 2009, 2011). As in yeast, different double mutants have different phenotypes that reveal different functional overlaps.

We describe here characterization of *Drosophila Fancm* mutants and comparison to *Blm* mutants. We show that FANCM has roles in preventing both mitotic and meiotic crossovers, independent of its function in the FA pathway, although the mitotic crossover frequency is lower in *Fancm* mutants than in *Blm* mutants. Similarly, *Fancm* mutants have a defect in SDSA repair of a gap, but it is significantly less severe than the defect in *Blm* mutants. Unlike *Blm* mutations, *Fancm* mutations are not synthetically lethal with single resolvase gene mutations; however, some combinations of multiple resolvase mutations are lethal to *Fancm* mutants. Finally, we show that spontaneous mitotic crossovers that occur in the absence of FANCM are dependent on MUS312 and either MUS81 or SLX1.

Materials and Methods

Drosophila stocks

Fly stocks were maintain at 25° with standard medium. Mutants were heteroallelic or hemizygous for null alleles (Table 1). Mutations in *Fancm* (*CG7922*) were found by TILLING (Cooper *et al.* 2008). $Fancm^{0693}$ is T-to-C substitution at 3R: 27,905,053 that generates a nonsense mutation (L78ter). In experiments reported here, *Fancm* mutants were $Fancm^{0693}/Df(3R)ED6058$. $Fancl^{LL00701}$ is an insertion of a *PBac{SAstopDsRed}* element into the boundary between the first intron and second exon between codons 37 and 38 (Schuldiner *et al.* 2008). The stock from the Drosophila Genetic Resource Center (Kyoto) had three *P* elements inserted onto other locations on the same chromosome arm; we

Table 1 Mutations used in this study

Allele	Type	Reference
Blm^{N1}	Deletion	McVey *et al.* (2007)
Blm^{D2}	Nonsense	Kusano *et al.* (2001)
Gen^{5997}	Frameshift	Andersen *et al.* (2011)
$mei\text{-}9^{a}$	Missense	Yıldız *et al.* (2004)
$mus312^{D1}$	Nonsense	Yıldız *et al.* (2002)
$mus312^{Z1973}$	Nonsense	Yıldız *et al.* (2002)
$mus81^{Nhe}$	Frameshift	Trowbridge *et al.* (2007)
$slx1^{F93I}$	Missense	Andersen *et al.* (2011)
$spn\text{-}A^{057}$	Missense	Staeva-Vieira *et al.* (2003)
$spn\text{-}A^{093A}$	Nonsense	Staeva-Vieira *et al.* (2003)

removed these by recombination before doing experiments with $Fancl^{LL00701}$. Alleles of other genes are listed in Table 1.

Sensitivity assays

Sensitivity to DNA-damaging agents was determined as in Yıldız *et al.* (2002). For HN2 and MMS, 250 μl of an aqueous solution at the indicated concentrations was added to the medium on which larvae were feeding. For ionizing radiation (IR), vials with larvae were exposed to gamma rays in an irradiator with 145 Ci of ^{137}Cs. Adults were counted daily from day 10 (after parents were first placed in vials) until day 18. Most treatments had at least three technical replicates (treatments on different days), each with 10 biological replications (different vials). Vials with <20 total progeny in either the untreated or the treated brood were discarded. Relative survival was calculated for each vial as the ratio between mutant and control flies in the treated vial, normalized to the same ratio in the untreated vial. To estimate absolute survival, we compared the number of control progeny in treated and untreated vials. For low doses, these were 81% (HN2), 82% (MMS), and 89% (IR). This reduced recovery is probably because flies in untreated vials were allowed to mate and lay eggs for 3 days, whereas flies in treated vials were allowed to lay eggs for only 2 days. Normalizing to these numbers, the highest doses had 81% (HN2), 56% (MMS), and 47% (IR) absolute survival. Statistical analyses were done in Prism 6 (GraphPad). For treatments that involved more than two genotypes, a Kruskal–Wallace test with a Dunn post-test was done. *P*-values reported are corrected for multiple comparisons. Treatments that involved only two genotypes were compared using an unpaired *t*-test.

Mitotic crossover assay

Mitotic crossovers in the male germline were measured as in McVey *et al.* (2007), using the genetic markers *st* and *Sb*. Each vial, each with a single male, was counted as a separate biological replicate. Vials with <20 progeny were discarded. Statistical analyses were done in Prism 6 (GraphPad). For treatments that involved more than two genotypes, a Kruskal–Wallace test with a Dunn post-test was done. *P*-values reported are corrected for multiple comparisons.

Meiotic crossover and nondisjunction assays

To measure meiotic crossovers, virgin females of the genotype *net* $dpp^{d\text{-}ho}$ *dp b pr cn* /+; $Fancm^{0693}$/*Df(3R)ED6058* (or wild-type control) were collected and aged 1–4 days and then crossed to *net* $dpp^{d\text{-}ho}$ *dp b pr cn* males. Progeny were counted and scored for each marker from day 10 to 14 after the cross was set up. To measure nondisjunction of the *X* chromosome, virgin females were crossed to males carrying *Dp(1;Y)*B^{S}, a *Y* chromosome carrying the B^{S} dominant marker. Normal progeny were females with wild-type eyes and males with Bar eyes; nondisjunctional progeny were females with Bar eyes (*XXY* progeny from *XX* ova or *XY* sperm) and males with wild-type eyes (*XO* progeny from nullo-*X* ova or nullo-*XY* sperm).

SDSA assay

The *P*{w^{a}} assay was done as described previously (McVey *et al.* 2004a), using the *CyO*, *H*{w^{+}, Δ2-3} transposase source. Because *Df(3R)ED6058* has a w^{+} allele associated with the deletion, the deletion chromosome was marked with *Sb*, and only *Sb*+ progeny were scored for eye color. A control was done with *Sb Df(3R)ED6058*/+ males; the results were not different from previous controls that did not have this *Df* chromosome.

Data archiving

Raw data have been deposited in the Carolina Digital Repository with the digital object identifier 10.15139/S3159M.

Results

Sensitivity of Fancm mutants to DNA-damaging agents

To investigate functions of *Drosophila* FANCM that are independent of the FA pathway, we compared phenotypes of *Fancm* mutants to those of *Fancl* mutants because FANCL is an essential component of the FA pathway but has no other known roles in DNA repair or recombination. Given the central function of the FA pathway in responding to ICLs, we first assayed sensitivity to a crosslinking agent, the nitrogen mustard mechlorethamine (HN2). *Fancl* mutants are hypersensitive to a high dose of HN2 (Figure 1A), consistent with a previous study that showed hypersensitivity to cross-linking agents after RNA interference knockdown of FANCL (Marek 2006). *Fancm* mutants were significantly more sensitive than *Fancl* mutants at this dose and were also hypersensitive to a lower dose of HN2, at which *Fancl* mutants were not hypersensitive.

The greater sensitivity of *Fancm* mutants suggests that FANCM has an FA-independent role in responding to ICLs or to another type of damage induced by HN2. Like most cross-linking agents, HN2 can induce mono-adducts and intrastrand cross-links in addition to ICLs (Wijen *et al.* 2000). We therefore assayed sensitivity to MMS, which generates mono-adducts but not cross-links (Beranek 1990). *Fancl* mutants were not hypersensitive to MMS at the doses

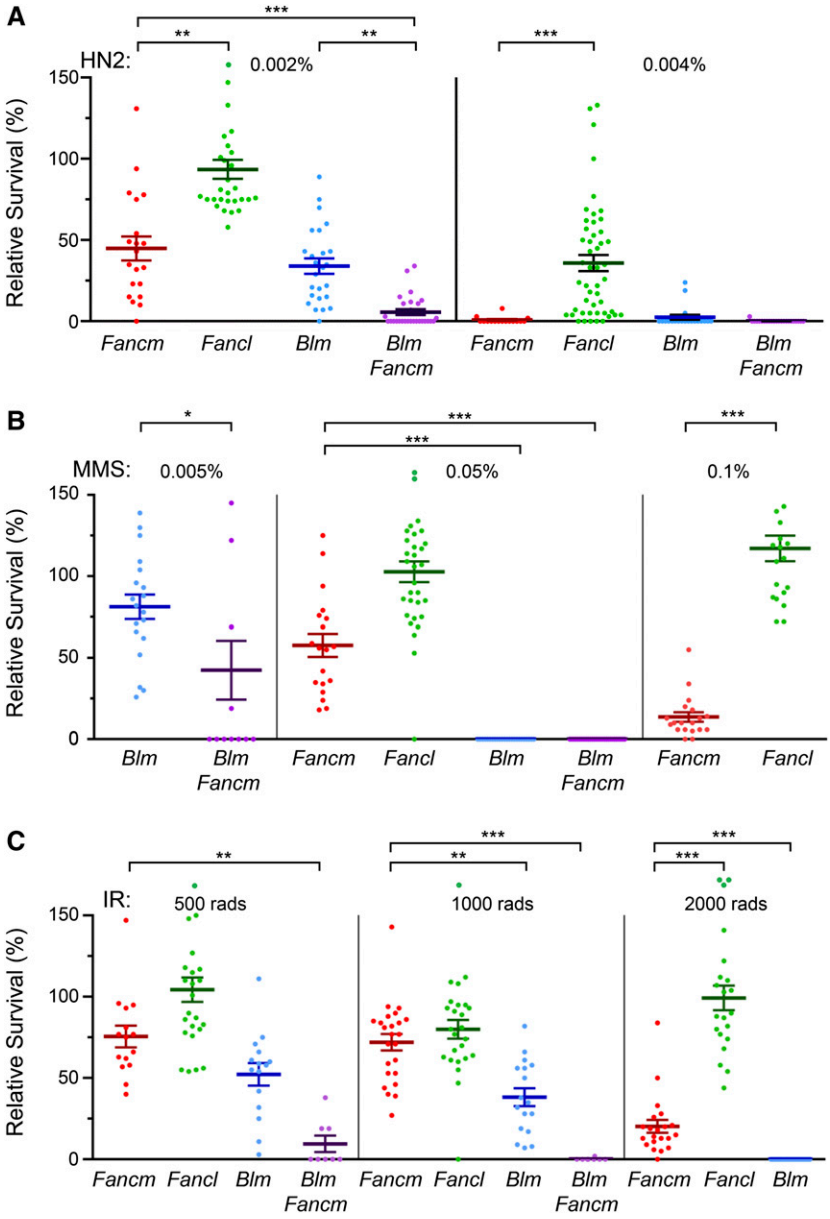

Figure 1 Comparison of sensitivities of *Fancm*, *Fancl*, and *Blm* mutants. Plots show survival of the indicated mutants relative to control flies in the same vial after exposure to (A) the nitrogen mustard mechloramine (HN2), (B) MMS, or (C) IR. Survival of control flies did not appear to be reduced at the lower doses used here, but the highest doses reduced survival of control flies by ~19% (HN2), 46% (MMS), and 53% (IR) (see *Materials and Methods*). Each dot represents one vial. Heavy bars are means; error bars are standard error of the mean. *n* = (left to right) HN2: 20, 30, 25, 28 | 16, 49, 20, 23; MMS: 26, 19 | 22, 32, 24, 28 | 19, 20; IR: 15, 25, 15, 8 | 24, 25, 17, 28 | 21, 21, 25. Statistical comparisons were done for *Fancm* compared to each other genotype, and *Fancm Blm* double mutants were compared to *Blm* single mutants: $*P < 0.05$; $**P < 0.01$, $***P < 0.001$ (corrected for multiple comparisons; see *Materials and Methods*); all statistically significant comparisons are indicated. Seven of the 625 data points, all from *Fancl* vials, are off the scale and are not shown (values in parentheses): 0.002 HN2 (1.92), 0.05% MMS (1.97), 0.1% MMS (1.84, 1.84, and 1.80), and 500 rad IR (1.92 and 1.76).

assayed, but *Fancm* mutants showed significant hypersensitivity to a high dose (Figure 1B). Studies in other model organisms revealed functions for FANCM orthologs in DSB repair pathways (Sun *et al.* 2008; Prakash *et al.* 2009; Crismani *et al.* 2012; Lorenz *et al.* 2012; Mazón and Symington 2013; Mitchel *et al.* 2013), so we also measured sensitivity to IR. *Fancm* mutants were hypersensitive to IR, but *Fancl* mutants were not, suggesting an FA-independent role for FANCM in DSB repair (Figure 1C).

Given the functional similarities between FANCM and BLM, we compared sensitivity between *Fancm* and *Blm* mutants. *Blm* mutants had about the same severity of hypersensitivity to HN2 as *Fancm* mutants, but were significantly more sensitive to MMS and to IR (Figure 1). *Blm Fancm* double mutants are fully viable in the absence of exogenous damage, but were more sensitive to MMS and HN2 than either single mutant. This suggests the existence of separate FANCM-dependent and BLM-dependent pathways for responding to base adduct damage and possibly to ICLs. Double mutants also appear to be more sensitive to IR than single mutants, but the difference between *Blm* and *Blm Fancm* is not statistically significant. This suggests that FANCM participates in a subset of the BLM-dependent responses to DSBs (*e.g.*, one of multiple branches that converge on or diverge from a BLM-dependent step). Vial-to-vial variation is often large in whole-animal assays such as this, especially at doses where survival is low, and this may have prevented us from detecting some real differences. If *Blm Fancm* double mutants are actually more sensitive to IR than *Blm* single mutants, it would suggest that FANCM and BLM contribute to different repair mechanisms, although this would not preclude overlap in the same mechanism.

Meiotic crossovers are elevated in some regions of the genome when FANCM is absent

S. pombe Fml1 and *Arabidopsis* FANCM suppress crossovers during meiotic recombination (Crismani *et al.* 2012; Knoll *et al.* 2012; Lorenz *et al.* 2012), and Fml1 and *S. cerevisiae* Mph1 suppress crossovers in vegetative cells (Sun *et al.* 2008; Prakash *et al.* 2009; Mazón and Symington 2013). We therefore assayed both meiotic and mitotic crossovers in *Drosophila Fancm* mutants. Meiotic crossovers were scored in five adjacent intervals spanning the tip of *2L* to the base of *2R*, a region comprising ~20% of the genome. The genetic distance across this region increased from 45.0 cM in wild-type females to 53.2 cM (118% of wild type) in *Fancm* mutants (Figure 2A; $P < 0.0001$). The increase is restricted to the two centromere-proximal intervals, each of which has about a threefold increase in crossovers compared to wild type (Figure 2, A and B). Double crossovers (DCOs) were also significantly more frequent in *Fancm* mutants: There were 44 DCOs among 2320 progeny (1.9%) from wild-type females, compared to 79 DCOs among 1484 progeny (5.3%) from *Fancm* ($P < 0.0001$). When progeny with multiple crossovers (DCOs and a small number with triple crossovers) are excluded, the crossover rates are not significantly different across the entire region assayed ($P = 0.1061$), but remain significantly elevated in the two proximal intervals ($P < 0.0001$ in each case; no significant differences in other intervals). Thus, in *Fancm* mutants, meiotic crossovers are elevated, but in only a subset of the genome.

This elevation does not appear to have any negative impact on chromosome segregation. In an assay for meiotic nondisjunction of the *X* chromosome, we detected one case of nondisjunction among 1698 progeny of wild-type females and one among 1592 progeny of *Fancm* mutant females ($P = 0.9636$ by χ^2 test).

Spontaneous mitotic crossovers are elevated when FANCM is absent

We assayed spontaneous mitotic crossovers in the male germline because there are no meiotic crossovers in males (Morgan 1912). We scored crossovers between the visible markers *st* and *Sb*, which are separated by >36 Mbp (~20% of the genome). Crossovers were not detected among progeny of wild-type males or *Fancl* mutant males, but were significantly elevated in *Fancm* mutant males (Figure 2C). In the same assay, the crossover frequency in *Blm* mutants is about threefold higher than in *Fancm* mutants. The rate in *Blm Fancm* double mutants was not significantly different from that in *Fancm* single mutants but was significantly lower than in *Blm* single mutants. A straightforward interpretation of this result is that FANCM functions upstream of BLM in a pathway that prevents mitotic crossovers; however, this is likely to be an oversimplification, given the multiple functions of these enzymes and the possibility of partial overlap in function (see *Discussion*).

A

Genotype	Genetic Distance (cM)						*n*
	net-dpp	*dpp-dp*	*dp-b*	*b-pr*	*pr-cn*	*net-cn*	
WT	5.1	7.5	27.3	3.5	1.5	45.0	2320
Fancm	5.1	6.0	27.5	10.2***	4.5***	53.2	1484

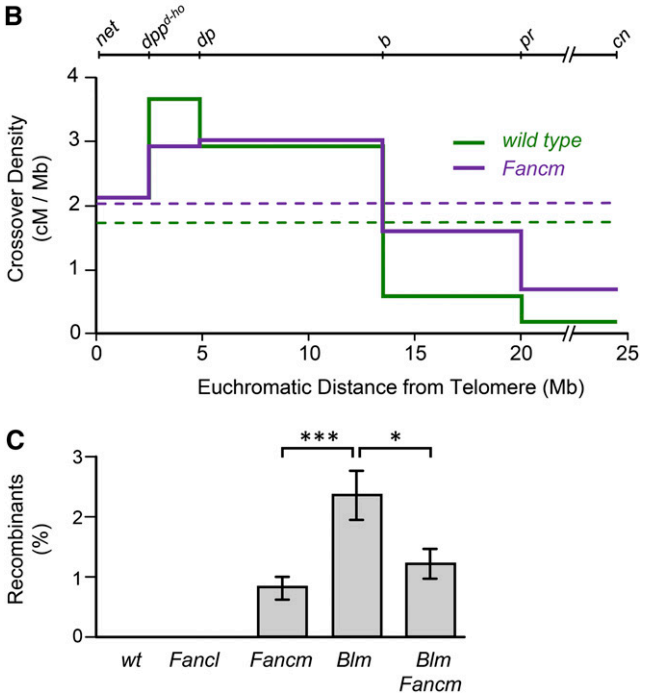

Figure 2 Meiotic and mitotic crossover elevation in *Fancm* mutants. (A) Genetic distances, in centimorgans, are given for five adjacent intervals on chromosome *2* (***$P < 0.0001$); other intervals were not significantly different ($P = 0.9647$, 0.0776, and 0.9406). (B) Meiotic crossover density. Data from (A) were graphed as crossover density, in centimorgans per megabase pair (Mb). The markers used are shown above the graph. Hash marks between *pr* and *cn* indicate the position of the centromere and pericentric heterochromatin (~16 Mb, not counted in distances shown). Solid lines depict density in each interval; dashed lines are mean density across the entire region. (C) Mitotic crossovers in the male germline. Bars show mean percentage of progeny that were recombinant between *st* and *Sb*. Error bars are standard error of the mean. No crossovers were detected in wild-type (wt) or *Fancl* mutant males. *Fancm*, *Blm*, and *Blm Fancm* were each significantly different from wild-type and *Fancl* ($P < 0.01$ for each comparison). The difference between *Fancm* and *Blm Fancm* was not significant ($P > 0.99$). *$P < 0.0294$; ***$P < 0.0001$. *P*-values reported have been adjusted for multiple comparisons (see *Materials and Methods*). *n* = (left to right) 40, 41, 46, 39, and 30.

FANCM has a modest role in SDSA

Hypersensitivity to IR and elevated mitotic crossovers suggest a role for FANCM in DSB repair, independent of its role in the FA pathway. It has been proposed that *S. pombe* Fml1 and *S. cerevisiae* Mph1 promote SDSA by disassembling D loops (Sun *et al.* 2008; Prakash *et al.* 2009; Mitchel *et al.* 2013), but this hypothesis has not been tested directly using an assay specific for SDSA. We used a gap repair assay in which products of SDSA can be distinguished from other types of repair (Adams *et al.* 2003; McVey *et al.* 2004a). A gap is generated by excision of a $P\{w^a\}$ element from the male *X* chromosome. This element carries the *apricot* allele of the *white* gene (w^a), in which a *copia*

retrotransposon is inserted into an intron, resulting in orange eye color instead of the wild-type red color (Figure 3B). Excision leaves a DSB that is repaired using the sister chromatid as a template. Since the sister still has an intact $P\{w^a\}$ element, this amounts to gap repair. Repair by two-ended SDSA can result in annealing between the long terminal repeats (LTRs) at the ends of *copia*, giving a product with only one LTR instead of an entire *copia*. Progeny that inherit this product have red eyes. This red-eyed class was decreased by ~50% in *Fancm* mutants compared to controls (Figure 3A), revealing a reduced ability to complete repair by SDSA. In *Blm* mutants, the decrease in SDSA is significantly more severe (Figure 3A) (McVey *et al.* 2007). Other types of repair result in loss of *white* gene function, which we recover as progeny with yellow eyes. In wild-type flies this is almost exclusively aborted SDSA in which there is templated synthesis from one or both ends of the gap followed by joining through an alternative end-joining pathway (Adams *et al.* 2003; McVey *et al.* 2004a,c; Chan *et al.* 2010). Molecular analyses of these repair products shows that synthesis tracts are significantly shorter in *Blm* mutants than in wild-type flies (Adams *et al.* 2003), but did not reveal any differences between wild-type and *Fancm* mutants (Figure 3B).

Both *Fancm* and *Blm* mutants have elevated spontaneous mitotic crossovers and a decreased ability to complete SDSA repair of a gap, but the defects in *Blm* mutants are more severe in both assays. Since *Fancm* is epistatic to *Blm* for mitotic crossovers and SDSA is thought to be an important pathway in crossover avoidance during DSB repair, we asked whether *Fancm* is epistatic to *Blm* in our SDSA assay. However, we were unable to generate any *Blm Fancm* double-mutant males carrying both the $P\{w^a\}$ element and transposase. This appeared to be due to recombination defects, since flies that also lacked the strand exchange protein Rad51 (*spn-A* mutants), and therefore are incapable of initiating recombination, do survive infrequently.

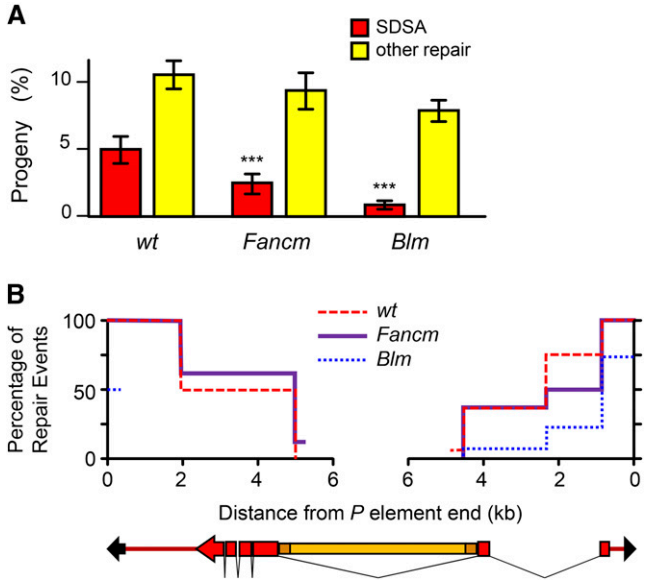

Figure 3 SDSA defects in *Fancm* mutants. (A) Gap repair outcomes. Bars show mean fraction of flies in each repair class: red bars indicate repair by SDSA, and yellow bars indicate other types of repair (usually aborted SDSA followed by end joining). Of the remaining flies, most came from cells that did not experience an excision event, although a small percentage may also be from the repair of the entire gap by SDSA. *Blm* data are from McVey *et al.* (2007). Error bars are standard error of the mean. $n = 85$ for *Fancm* and 45 for control. $^{***}P < 0.001$ compared to wild type. (B) Kaplan–Meier graph showing the amount of synthesis from each end of the *P* element in non-SDSA progeny (those with yellow eyes). PCR was done on sons of these progeny to detect synthesis from the left end at 5 bp, 1.7 kb, and 5.2 kb from the cut site and from the right end at 5 bp, 920 bp, 2.4 kb, and 4.6 kb from the cut site. *Blm* data are from Adams *et al.* (2003); the left end was analyzed only at 5 bp in that study. The drawing at the bottom represents the $P\{w^a\}$ element: black arrows, *P*-element ends; red, *white* gene (boxes, exons; lines, introns); orange, *copia* element (dark, long terminal repeats). $n = 16$ (*Fancm*), 83 (wild type), and 147 (*Blm*).

Spontaneous mitotic crossovers in the absence of FANCM require the scaffolding protein MUS312 and either MUS81 or SLX1

To better understand how spontaneous mitotic crossovers in *Fancm* mutants are generated, we asked whether any HJ resolvases are required. We made double mutants between *Fancm* and the genes encoding each of the catalytic subunits of the putative resolvases: *Gen*, *mus81*, *Slx1*, and *mei-9*. There was no significant difference in crossover frequency in any of these double mutants relative to *Fancm* single mutants (Figure 4A). However, crossovers were completely eliminated in double mutants with *mus312* (Figure 4A). MUS312 is a scaffolding protein that interacts physically and functionally with both MEI-9 (the *Drosophila* ortholog of Rad1/XPF) and SLX1 (Yıldız *et al.* 2002; Andersen *et al.* 2009), so we considered the possibility that SLX1 and MEI-9 have redundant roles in generating these mitotic crossovers in *Fancm* mutants. This does not appear to be the case because *mei-9*; *Slx1 Fancm* triple mutants have the same crossover rate as *Fancm* single mutants and both double mutants (Figure 4A). Orthologs of MUS312 from vertebrates (SLX4) and *C. elegans* (HIM-18) interact physically with MUS81 (Fekairi *et al.* 2009; Muñoz *et al.* 2009; Svendsen *et al.* 2009; Saito *et al.* 2013). A similar interaction has not been reported for the *Drosophila* proteins, and we failed to detect such an interaction in yeast two-hybrid assays (J. R. LaRocque and J. Sekelsky, personal communication). Nevertheless, we asked whether MUS81 and SLX1 might be acting redundantly to make mitotic crossovers in *Fancm* mutants. Crossovers were nearly absent in *mus81*; *Slx1 Fancm* triple mutants (Figure 4A), suggesting that MUS81 and SLX1 indeed act redundantly to make these crossovers and that both nucleases require MUS312 for this function.

Simultaneous loss of FANCM and multiple resolvases is lethal

In *Drosophila*, each of the three putative mitotic HJ resolvases (MUS81–MMS4, GEN, and MUS312–SLX1) are essential when BLM is absent (Trowbridge *et al.* 2007; Andersen *et al.* 2009, 2011). The experiments described above show that none of the resolvases are essential when FANCM is absent and that at least males are fertile (we did not assay female fertility since several of these resolvases are required

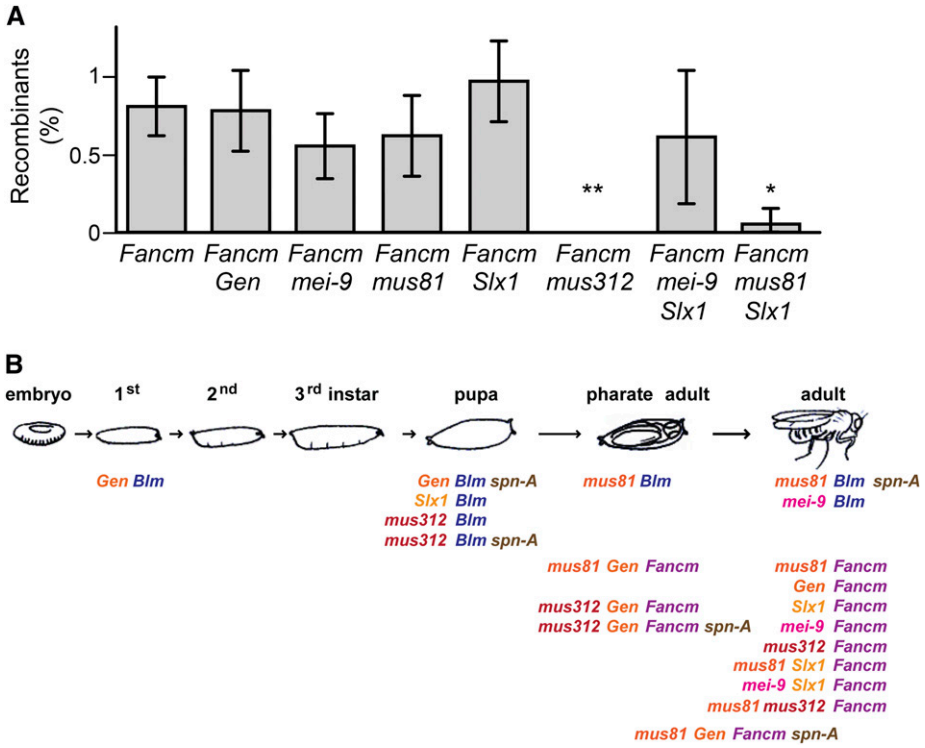

Figure 4 Phenotypes of *Fancm* mutants lacking one or more resolvases. (A) Mitotic crossovers in *Fancm* mutants lacking various resolvases. Error bars are standard error of the mean. Statistical significance was determined relative to *Fancm* single mutants (*P = 0.0298; **P = 0.0083). P-values reported have been adjusted for multiple comparisons (see *Materials and Methods*). n = (left to right) 46, 18, 18, 22, 17, 23, 20, and 22. (B) Lethality and viability of mutants lacking a helicase and one or more resolvases. At the top is a drawing of the developmental life cycle of *Drosophila*. Various genotypes lacking one of the anticrossover helicases (BLM or FANCM) and one or more of the putative resolvases are listed below the stage at which they die. Those listed below the adult live to adulthood. Life cycle stages and *Blm* mutant results are modified from Andersen *et al.* (2011).

for meiotic recombination and their absence causes high levels of nondisjunction and low fecundity). However, synthetic lethality was observed when certain combinations of resolvases were removed (Figure 4B). As in budding yeast (Blanco *et al.* 2010; Ho *et al.* 2010; Tay and Wu 2010), *Drosophila* MUS81–MMS4 and GEN have a partially redundant or compensatory relationship (Andersen *et al.* 2011). Simultaneous loss of both MUS81–MMS4 and GEN is lethal in *Fancm* mutants (Figure 4B). The *mus81*; *Gen Fancm* triple mutants survive to the pharate adult stage, which is the same stage at which *mus81*; *Blm* double mutants die but much later than lethality of *Gen Blm* double mutants, which only survive until the second instar larval stage. Preventing recombination partially suppresses *mus81*; *Blm* and *Gen Blm* lethality: *mus81*; *Blm spn-A* mutants are semiviable (~70% live to adulthood), and *Gen Blm spn-A* mutants survive to the pupal stage (Trowbridge *et al.* 2007; Andersen *et al.* 2011). To ask whether *mus81*; *Gen Fancm* inviability is similarly due to recombination defects, we made *mus81*; *Gen Fancm spn-A* quadruple mutants. A few quadruple mutants did survive to adulthood (4, compared to 84 expected), and these had rough eyes and cuticle defects suggestive of high rates of cell death during development. *Gen mus312 Fancm* triple mutants also died as pharate adult pupae; mutating *spn-A* had no apparent effect on this lethality (Figure 4B).

Discussion

Comparison of *Drosophila Fancm* and *Fancl* mutants for hypersensitivity to DNA-damaging agents (Figure 1) indicates that, as in fungi and plants, *Drosophila* FANCM has functions outside of the FA pathway. Among the agents that we tested, *Fancl* mutants were hypersensitive to only the cross-linking agent HN2, consistent with a primary or sole function for FANCL in the FA pathway. *Fancm* mutants were more sensitive to HN2 and, unlike *Fancl* mutants, were hypersensitive to the alkylating agent MMS and to ionizing radiation. Among these FA-independent roles, we focus on functions that may prevent crossovers.

FANCM in meiotic recombination

Drosophila Fancm mutants have a significant elevation in both meiotic and mitotic crossovers (Figure 2). Interestingly, the increase in meiotic crossovers was observed only in the two most proximal intervals, each of which had threefold more crossovers than wild-type females. Elevated meiotic crossovers have also been reported for *Arabidopsis FANCM* mutants, but in this case the elevation seems to be genome-wide (Crismani *et al.* 2012). The significance and cause of the elevation in *Drosophila* being restricted to only the two proximal intervals is unknown. One of these (*pr–cn*) includes the centromere and pericentric heterochromatin. It seems unlikely that the crossovers that we recovered occurred in heterochromatic regions since these are normally devoid of DSBs (Jang *et al.* 2003), but we did not directly determine whether the crossovers between *pr* and *cn* were in the euchromatic or heterochromatic portion of this interval.

Based on immunolocalization of meiotic recombination proteins, Knoll *et al.* (2012) hypothesized that *Arabidopsis* FANCM suppresses crossovers produced by MUS81, which is usually responsible for only 10–15% of meiotic crossovers in normal meiosis (Berchowitz *et al.* 2007). In *Drosophila*, most

meiotic crossovers are generated by a complex whose catalytic subunit is MEI-9, which is orthologous to XPF/Rad1 (Sekelsky *et al.* 1995). No role in generating meiotic crossovers has been detected for MUS81 (Trowbridge *et al.* 2007), so it will be interesting to determine whether the extra meiotic crossovers in *Fancm* mutants are dependent on MEI-9, MUS81, or another resolvase or combination of resolvases.

FANCM in synthesis-dependent strand annealing

Mitotic crossovers are elevated in the germlines of *Fancm* mutant males (Figure 2). Previous studies found elevated mitotic crossovers in *S. pombe fml1* mutants and in *S. cerevisiae mph1* mutants (Sun *et al.* 2008; Prakash *et al.* 2009; Mazón and Symington 2013; Mitchel *et al.* 2013). It is important to note that these studies in fungi involved enzymatic induction of DSBs, whereas the crossovers that we measured are spontaneous. Our experiments do not provide insight into the sources of these crossovers. FANCM may direct repair of spontaneous lesions toward noncrossover outcomes, or loss of FANCM may cause an increased incidence of some lesions, such as a DSB, that might be precursors to crossovers.

Mph1 and Fml1 have been proposed to promote DSB repair through the noncrossover SDSA pathway by disrupting D loops (Sun *et al.* 2008; Prakash *et al.* 2009; Tay *et al.* 2010; Mitchel *et al.* 2013). Hypersensitivity to ionizing radiation and elevated mitotic crossover frequency are consistent with *Drosophila* FANCM having a function in SDSA. In our gap repair assay for SDSA, we detected a 50% reduction in progeny with the phenotype diagnostic of completed SDSA repair, confirming a role for FANCM in this process (Figure 3). BLM/Sgs1 also has anticrossover functions during DSB repair. Although this is usually discussed in terms of the dHJ dissolution function, a function in SDSA is also apparent in the finding that Sgs1 generates meiotic noncrossovers (De Muyt *et al.* 2012) and that *Drosophila Blm* mutants are severely compromised in the $P\{w^a\}$ gap repair assay for SDSA (Adams *et al.* 2003). Molecular and genetic analysis of gap repair products from *Blm* mutants revealed that synthesis tracts are significantly shorter than in wild-type males, and deletions into flanking DNA sequences are significantly more frequent (Adams *et al.* 2003; McVey *et al.* 2007). It is thought that repair of the large gap in the $P\{w^a\}$ assay requires multiple cycles of strand exchange, synthesis, and D-loop disassembly (McVey *et al.* 2004a). This led to a model in which BLM is required for D-loop disassembly, and in the absence of BLM these structures are cut by a structure-selective endonuclease, leading to flanking deletions. This hypothesis raised the question of why there are synthesis tracts at all in *Blm* mutants. We propose that BLM-topoisomerase 3α is essential for disassembling D loops only after lengthy synthesis, but that FANCM can disassemble shorter D loops. Although some features of this model are attractive, it does not explain why there is an SDSA defect in *Fancm* mutants. It is also possible that both FANCM and BLM promote SDSA by disassembling D loops, but that they act at different stages of male germline development.

Resolvases in generating mitotic crossovers in Fancm mutants

Spontaneous mitotic crossovers that occur in the absence of FANCM require the MUS312 nuclease scaffold protein and either MUS81 or SLX1 (Figure 3A). One simple interpretation is that FANCM acts at an early stage to direct repair down a noncrossover pathway (*e.g.*, SDSA). In the absence of FANCM, an HJ-containing intermediate is generated, and it is the resolution of this intermediate by MUS81 or SLX1 that generates a crossover. Recent *in vitro* experiments suggest that vertebrate MUS81 and SLX1 collaborate to resolve HJs, with SLX1 making an initial nick and MUS81 making a second nick and both being coordinated by the MUS312 ortholog SLX4 (Castor *et al.* 2013; Garner *et al.* 2013; Wyatt *et al.* 2013). Our result is more consistent with MUS81 and SLX1 having redundant functions. If *Drosophila* MUS81 and SLX1 work together to resolve HJs, then it must not be loss of this activity that prevents crossovers when FANCM is absent.

The different phenotypes of *mus81* and *Slx1* mutants indicate that each enzyme has unique functions, but this does not preclude redundant or codependent functions (Trowbridge *et al.* 2007; Andersen *et al.* 2009). Both *S. cerevisiae* and human MUS81–EME1 and SLX4–SLX1 cut flap and replication fork structures *in vitro* (Fricke and Brill 2003; Ehmsen and Heyer 2008; Wyatt *et al.* 2013). It is possible that, rather than working late on a HJ intermediate, both MUS312–MUS81–MMS4 or MUS312–SLX1 can generate DSBs by cutting aberrant replication fork structures that would normally be processed by FANCM. Some of these DSBs may then be repaired through a pathway that results in a crossover with the homologous chromosome. Given the anticrossover roles of BLM during DSB repair, however, we might expect a synergistic effect on mitotic crossover frequency in *Blm Fancm* double mutants. Instead, we saw a crossover frequency more similar to that of *Fancm* single mutants. One weakness of this crossover assay is that we cannot detect complete failure of repair since this would likely result in apoptosis or spermatocyte defects. *Blm Fancm* males did not produce fewer progeny than single-mutant males, but we would not have been able to detect reductions of similar magnitude as the mitotic crossover frequency (∼2% in *Blm* single mutants).

We also found that *mus81 Gen Fancm* and *Gen mus312 Fancm* mutants are inviable. In the former of these genotypes, inviability may be explained by functional overlap between MSU81–MMS4 and GEN. *S. cerevisiae* Mus81–Mms4 and Yen1 exhibit partial redundancy, with Yen1 appearing to function primarily as a backup to Mus81–Mms4 (Blanco *et al.* 2010; Ho *et al.* 2010; Tay and Wu 2010). Redundancy has also been observed between *Drosophila* MUS81–MMS4 and GEN, although the relationship appears to be reversed, with *Gen* mutants having more

severe phenotypes than *mus81* mutants (Andersen *et al.* 2011). Inviability in *mus81 Gen Fancm* triple mutants is weakly suppressed by preventing recombination. This suggests that either the death of *mus81 Gen Fancm* mutants is only partially due to defects in recombination or that the alternatives to RAD51-mediated recombination are also detrimental.

No functional overlaps have been reported for GEN and MUS312–SLX1 or their orthologs. Triple mutants that lack GEN, MUS312, and FANCM may be inviable because of an accumulation of damage, some resulting from loss of GEN and FANCM and some resulting from loss of MUS312–SLX1 and FANCM. This lethality may also be in part due to loss of both GEN and those MUS81 activities that require MUS312 or some more complex interaction involving all three nucleases.

Concluding remarks

In summary, our analysis shows that *Drosophila Fancm* mutants have similar phenotypes to *Blm* mutants in several assays. These defects are generally less severe in *Fancm* mutants and more severe in *Blm Fancm* double mutants. This suggests that FANCM and BLM have overlapping functions and that these helicases are partially redundant or that either can partially compensate for loss of the other; however, several of the phenotypes that we assayed, such as viability after treatment with DNA-damaging agents, viability of flies carrying mutations in multiple genes, and mitotic crossing over, are fairly crude genetic readouts that might have several underlying causes. Thus, more detailed mechanistic studies will be necessary to tease apart the different cellular functions of BLM, FANCM, and the HJ resolvases.

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Literature Cited

Adams, M. D., M. McVey, and J. Sekelsky, 2003 *Drosophila* BLM in double-strand break repair by synthesis-dependent strand annealing. Science 299: 265–267.

Agostinho, A., B. Meier, R. Sonneville, M. Jagut, A. Woglar *et al.*, 2013 Combinatorial regulation of meiotic Holliday junction resolution in *C. elegans* by HIM-6 (BLM) helicase, SLX-4, and the SLX-1, MUS-81 and XPF-1 nucleases. PLoS Genet. 9: e1003591.

Andersen, S. L., and J. Sekelsky, 2010 Meiotic *vs.* mitotic recombination: two different routes for double-strand break repair: the different functions of meiotic *vs.* mitotic DSB repair are reflected in different pathway usage and different outcomes. BioEssays 32: 1058–1066.

Andersen, S. L., D. T. Bergstralh, K. P. Kohl, J. R. Larocque, C. B. Moore *et al.*, 2009 *Drosophila* MUS312 and the vertebrate ortholog BTBD12 interact with DNA structure-specific endonucleases in DNA repair and recombination. Mol. Cell 35: 128–135.

Andersen, S. L., H. K. Kuo, D. Savukoski, M. H. Brodsky, and J. Sekelsky, 2011 Three structure-selective endonucleases are essential in the absence of BLM helicase in *Drosophila*. PLoS Genet. 7: e1002315.

Bakker, S. T., H. J. Van De Vrugt, M. A. Rooimans, A. B. Oostra, J. Steltenpool *et al.*, 2009 *Fancm*-deficient mice reveal unique features of Fanconi anemia complementation group M. Hum. Mol. Genet. 18: 3484–3495.

Beranek, D. T., 1990 Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. Mutat. Res. 231: 11–30.

Berchowitz, L. E., K. E. Francis, A. L. Bey, and G. P. Copenhaver, 2007 The role of AtMUS81 in interference-insensitive crossovers in *A. thaliana*. PLoS Genet. 3: e132.

Blanco, M. G., J. Matos, U. Rass, S. C. Ip, and S. C. West, 2010 Functional overlap between the structure-specific nucleases Yen1 and Mus81-Mms4 for DNA-damage repair in *S. cerevisiae*. DNA Repair (Amst.) 9: 394–402.

Boddy, M. N., P. H. Gaillard, W. H. McDonald, P. Shanahan, J. R. Yates, III *et al.*, 2001 Mus81-Eme1 are essential components of a Holliday junction resolvase. Cell 107: 537–548.

Brosh, R.M., Jr., 2013 DNA helicases involved in DNA repair and their roles in cancer. Nat. Rev. Cancer 13: 542–558.

Castor, D., N. Nair, A. C. Declais, C. Lachaud, R. Toth *et al.*, 2013 Cooperative control of Holliday junction resolution and DNA repair by the SLX1 and MUS81–EME1 nucleases. Mol. Cell 52: 221–233.

Chan, S. H., A. M. Yu, and M. McVey, 2010 Dual roles for DNA polymerase theta in alternative end-joining repair of double-strand breaks in *Drosophila*. PLoS Genet. 6: e1001005.

Chen, X. B., R. Melchionna, C. M. Denis, P. H. Gaillard, A. Blasina *et al.*, 2001 Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. Mol. Cell 8: 1117–1127.

Cooper, J. L., B. J. Till, and S. Henikoff, 2008 Fly-TILL: reverse genetics using a living point mutation resource. Fly (Austin) 2: 300–302.

Crismani, W., C. Girard, N. Froger, M. Pradillo, J. L. Santos *et al.*, 2012 FANCM limits meiotic crossovers. Science 336: 1588–1590.

Dayani, Y., G. Simchen, and M. Lichten, 2011 Meiotic recombination intermediates are resolved with minimal crossover formation during return-to-growth, an analogue of the mitotic cell cycle. PLoS Genet. 7: e1002083.

De Los Santos, T., N. Hunter, C. Lee, B. Larkin, J. Loidl *et al.*, 2003 The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics 164: 81–94.

De Muyt, A., L. Jessop, E. Kolar, A. Sourirajan, J. Chen *et al.*, 2012 BLM helicase ortholog Sgs1 is a central regulator of meiotic recombination intermediate metabolism. Mol. Cell 46: 43–53.

Ehmsen, K. T., and W. D. Heyer, 2008 *Saccharomyces cerevisiae* Mus81-Mms4 is a catalytic, DNA structure-selective endonuclease. Nucleic Acids Res. 36: 2182–2195.

Fekairi, S., S. Scaglione, C. Chahwan, E. R. Taylor, A. Tissier *et al.*, 2009 Human SLX4 is a Holliday junction resolvase subunit

that binds multiple DNA repair/recombination endonucleases. Cell 138: 78–89.
Fricke, W. M., and S. J. Brill, 2003 Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. Genes Dev. 17: 1768–1778.
Gari, K., C. Décaillet, A. Z. Stasiak, A. Stasiak, and A. Constantinou, 2008 The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. Mol. Cell 29: 141–148.
Garner, E., Y. Kim, F. P. Lach, M. C. Kottemann, and A. Smogorzewska, 2013 Human GEN1 and the SLX4-associated nucleases MUS81 and SLX1 are essential for the resolution of replication-induced Holliday junctions. Cell Reports 5: 207–215.
Ho, C. K., G. Mazon, A. F. Lam, and L. S. Symington, 2010 Mus81 and Yen1 promote reciprocal exchange during mitotic recombination to maintain genome integrity in budding yeast. Mol. Cell 40: 988–1000.
Ip, S. C., U. Rass, M. G. Blanco, H. R. Flynn, J. M. Skehel *et al.*, 2008 Identification of Holliday junction resolvases from humans and yeast. Nature 456: 357–361.
Jang, J. K., D. E. Sherizen, R. Bhagat, E. A. Manheim, and K. S. McKim, 2003 Relationship of DNA double-strand breaks to synapsis in Drosophila. J. Cell Sci. 116: 3069–3077.
Kaliraman, V., J. R. Mullen, W. M. Fricke, S. A. Bastin-Shanower, and S. J. Brill, 2001 Functional overlap between Sgs1-Top3 and the Mms4-Mus81 endonuclease. Genes Dev. 15: 2730–2740.
Karow, J. K., A. Constantinou, J. L. Li, S. C. West, and I. D. Hickson, 2000 The Bloom's syndrome gene product promotes branch migration of Holliday junctions. Proc. Natl. Acad. Sci. USA 97: 6504–6508.
Kim, H., and A. D. D'Andrea, 2012 Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev. 26: 1393–1408.
Knoll, A., J. D. Higgins, K. Seeliger, S. J. Reha, N. J. Dangel *et al.*, 2012 The Fanconi anemia ortholog FANCM ensures ordered homologous recombination in both somatic and meiotic cells in *Arabidopsis*. Plant Cell 24: 1448–1464.
Kohl, K. P., C. D. Jones, and J. Sekelsky, 2012 Evolution of an MCM complex in flies that promotes meiotic crossovers by blocking BLM helicase. Science 338: 1363–1365.
Kusano, K., D. M. Johnson-Schlitz, and W. R. Engels, 2001 Sterility of Drosophila with mutations in the Bloom syndrome gene: complementation by Ku70. Science 291: 2600–2602.
Lorenz, A., F. Osman, W. Sun, S. Nandi, R. Steinacher *et al.*, 2012 The fission yeast FANCM ortholog directs non-crossover recombination during meiosis. Science 336: 1585–1588.
Marek, L. R., and A. E. Bale, 2006 Drosophila homologs of FANCD2 and FANCL function in DNA repair. DNA Repair (Amst) 5: 1317–1326.
Mazón, G., and L. S. Symington, 2013 Mph1 and Mus81-Mms4 prevent aberrant processing of mitotic recombination intermediates. Mol. Cell 52: 63–74.
McVey, M., M. D. Adams, E. Staeva-Vieira, and J. J. Sekelsky, 2004a Evidence for multiple cycles of strand invasion during repair of double-strand gaps in Drosophila. Genetics 167: 699–705.
McVey, M., J. R. Larocque, M. D. Adams, and J. J. Sekelsky, 2004b Formation of deletions during double-strand break repair in *Drosophila* DmBlm mutants occurs after strand invasion. Proc. Natl. Acad. Sci. USA 101: 15694–15699.
McVey, M., D. Radut, and J. Sekelsky, 2004c End-joining repair of double-strand breaks in Drosophila is largely DNA ligase IV-independent. Genetics 168: 2067–2076.
McVey, M., S. L. Andersen, Y. Broze, and J. Sekelsky, 2007 Multiple functions of Drosophila BLM helicase in maintenance of genome stability. Genetics 176: 1979–1992.
Mitchel, K., K. Lehner, and S. Jinks-Robertson, 2013 Heteroduplex DNA position defines the roles of the Sgs1, Srs2, and Mph1 helicases in promoting distinct recombination outcomes. PLoS Genet. 9: e1003340.
Morgan, T. H., 1912 Complete linkage in the second chromosome of the male of *Drosophila*. Science 36: 719–720.
Mosedale, G., W. Niedzwiedz, A. Alpi, F. Perrina, J. B. Pereira-Leal *et al.*, 2005 The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. Nat. Struct. Mol. Biol. 12: 763–771.
Muñoz, I. M., K. Hain, A. C. Declais, M. Gardiner, G. W. Toh *et al.*, 2009 Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. Mol. Cell 35: 116–127.
Osman, F., J. Dixon, C. L. Doe, and M. C. Whitby, 2003 Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. Mol. Cell 12: 761–774.
Prakash, R., D. Satory, E. Dray, A. Papusha, J. Scheller *et al.*, 2009 Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. Genes Dev. 23: 67–79.
Saito, T. T., D. Y. Lui, H.-M. Kim, K. Meyer, and M. P. Colaiácovo, 2013 Interplay between structure-specific endonucleases for crossover control during *Caenorhabditis elegans* meiosis. PLoS Genet. 9: e1003586.
Schuldiner, O., D. Berdnik, J. M. Levy, J. S. Wu, D. Luginbuhl *et al.*, 2008 *piggyBac*-based mosaic screen identifies a postmitotic function for cohesin in regulating developmental axon pruning. Dev. Cell 14: 227–238.
Schwartz, E. K., and W. D. Heyer, 2011 Processing of joint molecule intermediates by structure-selective endonucleases during homologous recombination in eukaryotes. Chromosoma 120: 109–127.
Sekelsky, J. J., K. S. McKim, G. M. Chin, and R. S. Hawley, 1995 The Drosophila meiotic recombination gene *mei-9* encodes a homologue of the yeast excision repair protein Rad1. Genetics 141: 619–627.
Soulier, J., 2011 Fanconi anemia. Hematology Am. Soc. Hematol. Educ. Program 2011: 492–497.
Staeva-Vieira, E., S. Yoo, and R. Lehmann, 2003 An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. EMBO J. 22: 5863–5874.
Sun, W., S. Nandi, F. Osman, J. S. Ahn, J. Jakovleska *et al.*, 2008 The FANCM ortholog Fml1 promotes recombination at stalled replication forks and limits crossing over during DNA double-strand break repair. Mol. Cell 32: 118–128.
Svendsen, J. M., A. Smogorzewska, M. E. Sowa, B. C. O'Connell, S. P. Gygi *et al.*, 2009 Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. Cell 138: 63–77.
Tay, Y. D., and L. Wu, 2010 Overlapping roles for Yen1 and Mus81 in cellular Holliday junction processing. J. Biol. Chem. 285: 11427–11432.
Tay, Y. D., J. M. Sidebotham, and L. Wu, 2010 Mph1 requires mismatch repair-independent and -dependent functions of MutSalpha to regulate crossover formation during homologous recombination repair. Nucleic Acids Res. 38: 1889–1901.
Trowbridge, K., K. S. McKim, S. Brill, and J. Sekelsky, 2007 Synthetic lethality in the absence of the Drosophila MUS81 endonuclease and the DmBlm helicase is associated with elevated apoptosis. Genetics 176: 1993–2001.
Van Brabant, A. J., T. Ye, M. Sanz, I. J. German, N. A. Ellis *et al.*, 2000 Binding and melting of D-loops by the Bloom syndrome helicase. Biochemistry 39: 14617–14625.
Wechsler, T., S. Newman, and S. C. West, 2011 Aberrant chromosome morphology in human cells defective for Holliday junction resolution. Nature 471: 642–646.

Whitby, M. C., 2005 Making crossovers during meiosis. Biochem. Soc. Trans. 33: 1451–1455.
Whitby, M. C., 2010 The FANCM family of DNA helicases/translocases. DNA Repair (Amst.) 9: 224–236.
Wijen, J. P., M. J. Nivard, and E. W. Vogel, 2000 The in vivo genetic activity profile of the monofunctional nitrogen mustard 2-chloroethylamine differs drastically from its bifunctional counterpart mechlorethamine. Carcinogenesis 21: 1859–1867.
Wu, L., and I. D. Hickson, 2003 The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 426: 870–874.
Wyatt, H. D., S. Sarbajna, J. Matos, and S. C. West, 2013 Coordinated actions of SLX1–SLX4 and MUS81–EME1 for Holliday junction resolution in human cells. Mol. Cell 52: 234–247.
Yıldız, Ö., S. Majumder, B. C. Kramer, and J. Sekelsky, 2002 Drosophila MUS312 interacts with the nucleotide excision repair endonuclease MEI-9 to generate meiotic crossovers. Mol. Cell 10: 1503–1509.
Yıldız, Ö., H. Kearney, B. C. Kramer, and J. J. Sekelsky, 2004 Mutational analysis of the Drosophila DNA repair and recombination gene *mei-9*. Genetics 167: 263–273.

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