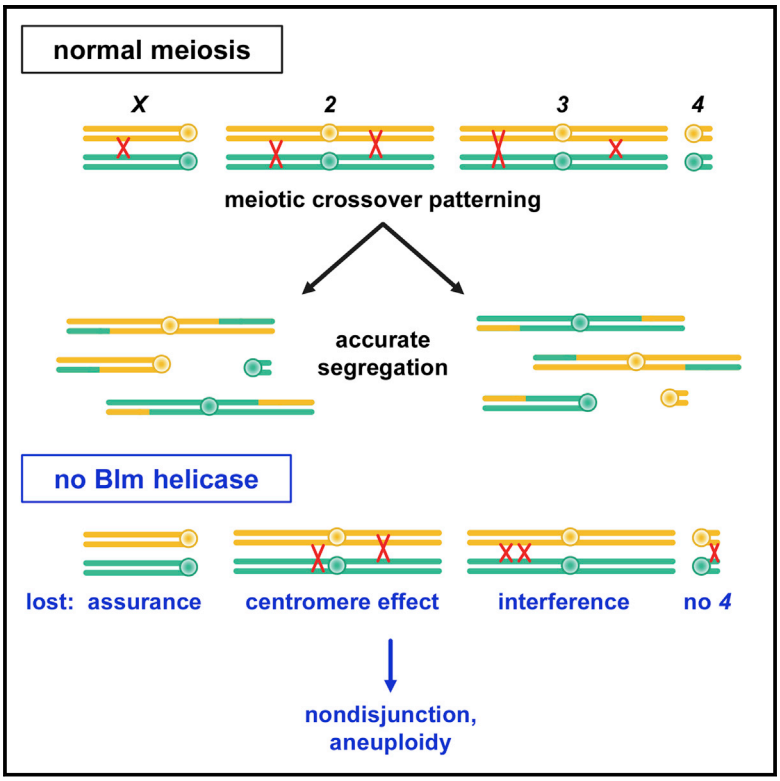
Report

Current Biology

Bloom Syndrome Helicase Promotes Meiotic Crossover Patterning and Homolog Disjunction

Graphical Abstract



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In Brief

Hatkevich et al. demonstrate that the *Drosophila* Bloom syndrome helicase chaperones double-strand break intermediates into the meiotic recombination pathway. In *Blm* mutants, the crossover/noncrossover decision is unregulated, leading to the loss of crossover patterning and increased nondisjunction.

Highlights

- Blm is essential for the primary meiotic recombination pathway in *Drosophila*
- In *Blm* mutants, interference and other types of crossover patterning are lost
- In *Blm* mutants, meiotic crossovers are made on chromosome *4*
- Loss of regulated crossover designation leads to elevated nondisjunction

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Bloom Syndrome Helicase Promotes Meiotic Crossover Patterning and Homolog Disjunction

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SUMMARY

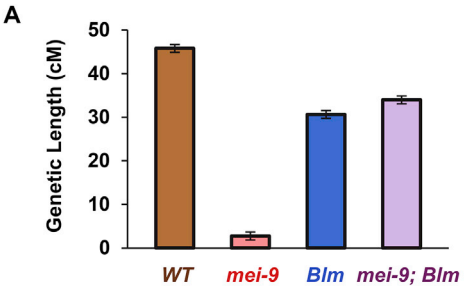
In most sexually reproducing organisms, crossover formation between homologous chromosomes is necessary for proper chromosome disjunction during meiosis I. During meiotic recombination, a subset of programmed DNA double-strand breaks (DSBs) are repaired as crossovers, with the remainder becoming noncrossovers [1]. Whether a repair intermediate is designated to become a crossover is a highly regulated decision that integrates several crossover patterning processes, both along chromosome arms (interference and the centromere effect) and between chromosomes (crossover assurance) [2]. Because the mechanisms that generate crossover patterning have remained elusive for over a century, it has been difficult to assess the relationship between crossover patterning and meiotic chromosome behavior. We show here that meiotic crossover patterning is lost in *Drosophila melanogaster* mutants that lack the Bloom syndrome helicase. In the absence of interference and the centromere effect, crossovers are distributed more uniformly along chromosomes. Crossovers even occur on the small chromosome *4*, which normally never has meiotic crossovers [3]. Regulated distribution of crossovers between chromosome pairs is also lost, resulting in an elevated frequency of homologs that do not receive a crossover, which in turn leads to elevated nondisjunction.

RESULTS AND DISCUSSION

Crossover interference, discovered by Sturtevant more than 100 years ago [4], is a meiotic crossover patterning phenomenon in which the presence of a crossover in one interval reduces the probability of a crossover in an adjacent interval [5]. Studies in budding yeast, *Arabidopsis*, and mice revealed a subset of meiotic crossovers that do not show interference [5]. These "class II crossovers" are generated through a different pathway than most (class I) meiotic crossovers [6]. In budding yeast, single-locus hotspot assays show that meiotic crossovers generated in the absence of the Bloom syndrome helicase (Blm) ortholog Sgs1 are formed primarily or exclusively by the class II pathway [7, 8]. This conclusion was partially derived from the observation that crossovers formed in *sgs1* meiotic null mutants are not dependent upon Mlh1, a component of the meiosis-specific, class I Holliday junction resolvase. We asked whether *Drosophila* Blm is also required to populate the class I pathway by determining whether crossovers generated in the absence of Blm are dependent upon MEI-9, the catalytic subunit of the presumptive *Drosophila* meiotic resolvase [9]. We measured crossovers in five adjacent intervals spanning most of *2L* and part of *2R* (for simplicity, referred to as *2L*), a region comprising ~20% of the euchromatic genome. As in previous studies [10], loss of MEI-9 resulted in a >90% reduction in crossovers compared to wild-type flies (Figure 1A). While *Blm* single mutants exhibit an ~30% decrease in crossovers on *2L* [11], there is no additional reduction of crossovers in *mei-9*; *Blm* double mutants. Therefore, the crossovers that occur in *Blm* mutants do not require MEI-9, suggesting that they are generated through the class II pathway.

The original distinction between crossovers generated by the class I and class II pathways is that only the former exhibit crossover interference [12]. We measured crossovers in three adjacent intervals on the X chromosome and calculated Stevens' [13] measure of interference (I = 1 − [observed double crossovers/expected double crossovers]) between pairs of intervals. Interference was strong in wild-type flies (I = 0.89 and 0.85 for the two pairs of adjacent intervals) but was significantly reduced in *Blm* mutants (I = 0.19 and 0.29, Figure 1B). Thus, without Blm helicase, crossovers are not dependent on the class I resolvase MEI-9, and interference among these crossovers is severely reduced or absent. This demonstrates that, as in *S. cerevisiae*, *Drosophila* Blm is required for the generation of crossovers through the class I pathway.

Given the loss of interference, we asked whether another important process that patterns crossovers along chromosomes arms—the centromere effect—is also lost in *Blm* mutants. This phenomenon, first reported by Beadle in 1932 [14], is the suppression of crossover formation in centromere-proximal



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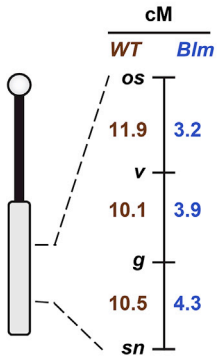

Genotype	DCOs Exp.	DCOs Obs.	Interference (*I*)	*P*
WT	38	4	0.89	0.0129
Blm	6	5	0.19	
WT	33	5	0.85	0.0277
Blm	9	7	0.29	

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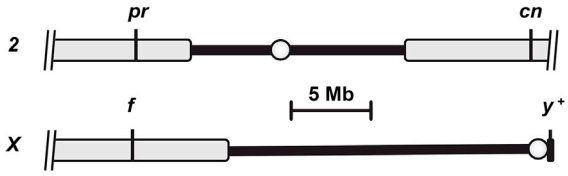


Chr.	Geno.	Crossovers exp.	Crossovers obs.	Cen. Effect (*CE*)	*P*
2	*WT*	695	73	0.89	< 0.0001
	Blm	118	75	0.36	
X	*WT*	371	249	0.29	0.0143
	Blm	84	87	-0.04	

Figure 1. Crossover Patterning Is Lost in *Blm* Mutants

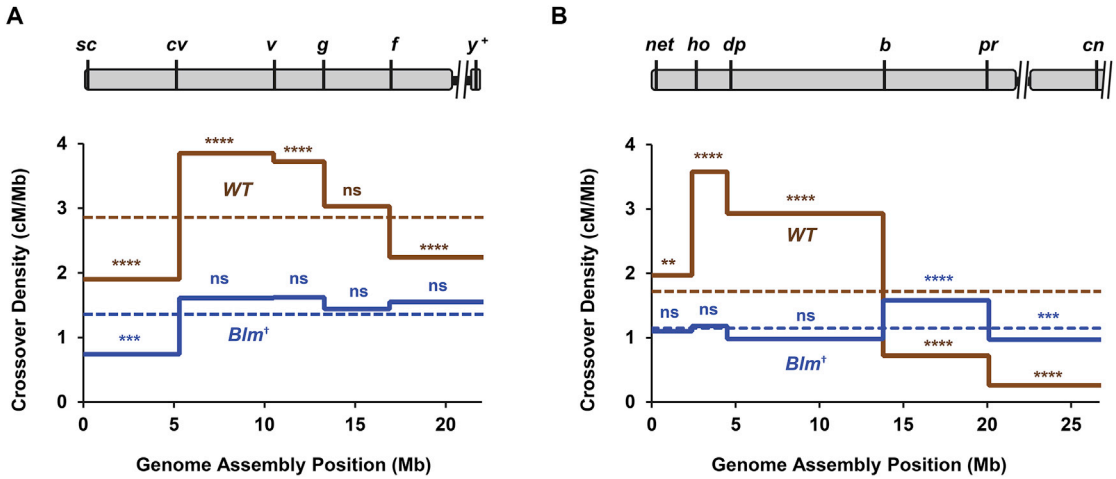
(A) Genetic length of the *net-cn* region in different genotypes. Bars are ±95% confidence intervals. Genotypes were wild-type (WT; n = 4,222), *mei-9* (n = 2,433), *Blm* (n = 1,171), and *mei-9*; *Blm* (n = 1,074). For the entire dataset, refer to Figure S1 and Table S1.

(B) X chromosome interference. Crossovers were measured in three adjacent intervals in the middle of the X, as in the schematic (circle, centromere; black line, unassembled pericentromeric satellite sequences; gray, genome assembly). To the right of the schematic, genetic lengths of these intervals in wild-type flies (n = 3,088) and *Blm* mutants (n = 4,953) are shown. Stevens' [13] measurement of interference (*I*) was calculated as follows: 1 − (observed double crossovers/expected double crossovers); *I* = 1 indicates complete interference and *I* = 0 is no interference. DCOs, double crossovers. For the complete dataset, refer to Table S2.

(C) The centromere effect. Schematics show the centromere-spanning intervals assayed. Lines are as in (B). The table below shows *CE* values for wild-type flies and *Blm* mutants in centromere-spanning intervals of *2* and X. *CE* values were calculated as 1 − (observed crossovers/expected crossovers) in proximal *f-y*$^{+}$ and *pr-cn* intervals. Expected was calculated as: total crossovers * (length of proximal interval/total length). Two-tailed Fisher's exact test was used to determine the significance between observed and expected crossover values between specified genotypes. For the entire dataset, refer to Tables S1 and S3.

euchromatin. To quantify the centromere effect, we devised a measure, *CE*, that is analogous to *I* as a measure of interference in that *CE* = 1 − (observed/expected), where observed is the number of crossovers counted in the interval and expected is the number expected in a random distribution (see the Supplemental Information for more details). In wild-type females, the interval between *pr* and *cn*, which spans the chromosome *2* centromere, had a *CE* of 0.89, consistent with a strong centromere effect (Figure 1C). In *Blm* mutants this was reduced to 0.36 ($p < 0.0001$). The centromere effect was much weaker on the X chromosome due to the larger block of heterochromatin that moves the euchromatin further from the centromere [15, 16] (Figure 1C). *CE* in a centromere-spanning interval on the X was 0.29 in wild-type flies, but it was reduced to −0.04 in *Blm* mutants ($p = 0.0143$).

Loss of interference and the centromere effect in *Blm* mutants allows us to assess the consequences of loss of crossover patterning along chromosome arms. Because these crossover patterning processes are responsible for the overall crossover distribution along each chromosome arm [17], we first assessed the effect of these losses on crossover distribution along entire arms. In wild-type flies, genetic length was not proportional to physical length, with crossover density being higher in the middle of each arm [17, 18]. On both the X and *2L*, crossover distribution in *Blm* mutants was significantly different from the wild-type distribution ($p = 0.0009$ and $p < 0.0001$, respectively) (Figures 2A and 2B). Instead, crossovers in *Blm* mutants appeared to be distributed in a manner more proportional to physical length. In wild-type flies, nine of the ten intervals we examined had significantly different numbers of crossovers than expected if genetic distance is proportional to physical distance, but in *Blm* mutants only three intervals were significantly different than this expectation (Figures 2A and 2B). The deviations in three intervals in *Blm* mutants may reflect residual crossover patterning; however, the *2L* crossover distributions in *mei-9*; *Blm* double mutants (Figure S1A) and in mutants carrying the helicase-dead allele *Blm*^{E866K} (Figure S1C) were even more closely proportional to physical length, suggesting that the departures from proportionality in *Blm*-null mutants may be an effect of strain background.



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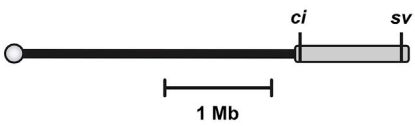

Geno.	COs	*n*	*P*
WT	0	3112	0.001
Blm	10	3106	

Figure 2. Intrachromosomal Effects of the Loss of Crossover Patterning

(A) Crossover distribution on the X chromosome. Schematic at the top is as in Figure 1 except that unassembled satellite sequences and the centromere are not included in density calculations. Locations of markers used to score crossovers are indicated. The graph below shows crossover density in each interval in wild-type flies (n = 2,179) and in *Blm* mutants (n = 1,099). Dotted line is mean density across the entire region assayed. Indicators of statistical significance are for chi-square tests on the observed number of crossovers versus the expected number if crossover number is proportional to physical size (ns, $p > 0.05$; ***$p < 0.001$; and ****$p < 0.0001$ after correction for multiple comparisons; $^{\dagger}p < 0.001$ indicates significance of *Blm* distribution across all intervals as compared to wild-type, as determined by G test of goodness of fit). For the complete dataset, refer to Table S3.

(B) Crossover distribution on *2L*. Schematic and graph are as in (A) (n = 4,222 for wild-type and 1,171 for *Blm*; **$p < 0.003$ and $^{\dagger}p < 0.0001$ for overall distribution in *Blm* mutants compared to wild-type, G test). For the complete dataset, refer to Table S1. Transposable elements were excluded from physical lengths in these analyses. See Figure S4 for details.

(C) Crossovers on chromosome *4*. Schematic is as in (A) and (B) but the scale is different. The table below shows the number of flies scored and the number of crossovers detected. For details on parental and recombinant classes, please refer to Table S4. Crossover density on the X, *2L*, and *4* are shown in Figure S2A.

We next examined a particularly extreme case of crossover patterning: the absence of crossovers on the small chromosome *4* of *Drosophila melanogaster*. There are never crossovers on this chromosome in wild-type females [3], but there have been reports of conditions that do result in crossovers. Grell [19] induced crossovers on *4* through heat shock, but it is not known whether these were meiotic or mitotic. Sandler and Szauter [20] observed crossovers in *mei-218* mutants, but others were unable to repeat this [17]. Osborne [21] found crossovers in *4*-derived sequences when they were translocated to chromosome *3*. This result suggests that the absence of crossovers on *4* may be a consequence of crossover patterning processes. Support for this idea came from whole-genome sequencing that revealed the presence of noncrossover gene conversion on *4* [22], indicating that double-strand breaks (DSBs) are made on *4* and, therefore, it is the repair process that is regulated to prevent crossovers.

We scored recombination between two markers near opposite ends of the genome sequence assembly of *4* (Figure 2C). As expected, we did not recover any crossovers between these markers in wild-type females (n = 3,112 progeny); however, in *Blm* mutants, we recovered ten crossovers among 3,106 progeny ($p = 0.001$, two-tailed Fisher's exact test). *Blm* mutants have spontaneous mitotic crossovers in the male germline [23]. To ensure that the crossovers we observed were meiotic, we eliminated meiotic DSBs; we did not observe any crossovers in this case (Tables S1 and S2). We conclude that the absence of crossovers on chromosome *4* in wild-type females is a result of active meiotic crossover patterning processes that are intertwined with the class I crossover pathway. This is most likely due to the centromere effect, consistent with the observation that crossovers occur in *4* sequences that are translocated to a genomic region further from the centromere [21]. Interference should not be applicable to *4* because there are no initial crossover designations to discourage nearby additional designations.

Although crossovers in *Blm* mutants were distributed approximately evenly along X and *2L*, and also occurred on chromosome

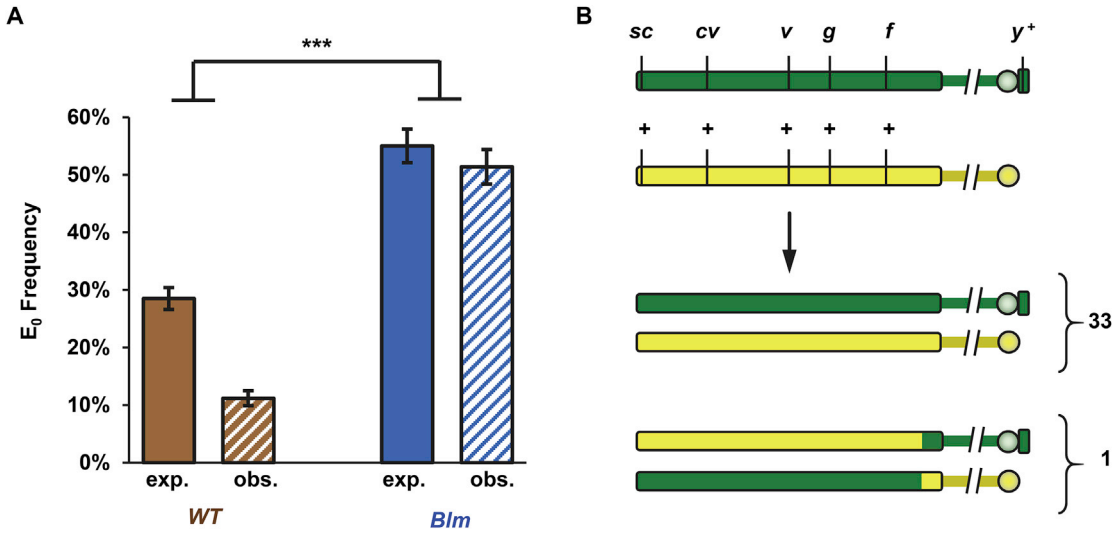

Figure 3. Loss of Interchromosomal Crossover Patterning in *Blm* Mutants

(A) The expected (based on Poisson distribution) and observed frequencies of E_0 X chromosomes in wild-type and in *Blm* mutants. Bars are 95% confidence intervals (***$p < 0.0001$ based on two-tailed Fisher's exact test of numbers observed and expected). For a similar analysis of chromosome *2L*, please see Figure S3.

(B) Classification of nondisjoined chromosomes. The top schematic represents heterozygous X chromosomes in *Blm* mutant females. Below are structures of chromosomes recovered in daughters that inherited two maternal X chromosomes. All were meiosis I nondisjunction based on the centromere-linked marker (y^+); 33 were non-recombinant; one had a crossover between *f* and y^+ (arbitrarily drawn within the euchromatin). The frequency of non-exchange (E_0) X chromosomes among those that failed to disjoin (0.97) is significantly different than among those X chromosomes that disjoined correctly (0.51) ($p = 0.0005$).

4, average crossover density was not the same between these chromosomes (Figure S2A). In both wild-type females and *Blm* mutants, crossover density was higher on the X than on *2L*, and it was lower still on chromosome *4* in *Blm* mutants. Possible explanations for this include different DSB densities, different strengths of crossover patterning (e.g., the weaker centromere effect on the X compared to chromosome *2*), and residual crossover patterning in *Blm* mutants.

The results above show that crossover patterning along chromosomes is lost or severely reduced in *Blm* mutants. Crossover patterning also occurs between chromosomes. Darlington and Dark [24] reported that, in a grasshopper species with a large range of chromosome sizes, every pair of homologous chromosomes always had at least one chiasma (the cytological manifestation of a crossover), called the obligate chiasma [25]. The occurrence of an obligate chiasma suggests that there is an active process, referred to as crossover assurance, that monitors the designation of crossovers on each chromosome. To determine whether loss of Blm affects crossover assurance, we compared the observed and expected frequencies of E_0 tetrads (homologous chromosome pairs with no crossovers). In wild-type flies, the observed E_0 frequency for the X chromosome (0.112) was less than half the frequency expected based on Poisson distribution (0.285, $p < 0.0001$) (Figure 3A), indicating that crossover assurance is present, but it is not absolute. Crossover assurance is significantly reduced or absent in *Blm* mutants ($p < 0.0001$ compared to wild-type), resulting in the observed E_0 frequency (0.514) being similar to the expected frequency (0.550).

The results described above reveal that the three major aspects of crossover patterning that occur along and among chromosomes—interference, the centromere effect, and assurance—are significantly decreased or eliminated when Blm helicase is absent. This suggests an inability to make or execute the crossover/noncrossover decision. Mapping of noncrossover gene conversion events in wild-type flies through whole-genome sequencing [22, 26] reveals a flat distribution along each of the major chromosome arms, similar to the distribution of crossovers in *Blm* mutants (Figures 2 and S2). Miller et al. [26] showed that noncrossovers do not participate in interference and are not subject to the centromere effect. These findings suggest that DSBs are evenly distributed along each arm, at least at the megabase scales at which we mapped crossovers. In wild-type flies, crossover patterning processes act on this distribution such that events in the central regions of the major chromosome arms have a higher probability of being designated to become crossovers, and those on chromosome *4* are never so designated. Regulated crossover designation is lost in *Blm* mutants, and, as a result, every DSB repair event has the same probability of becoming a crossover, regardless of where along the chromosome it is located.

Our results argue that meiotic DSB repair in *Blm* mutants occurs outside of the predominant meiotic recombination pathway and that this results in the loss of regulated crossover designation and patterning. What are the consequences of these losses on meiosis? In *Blm* mutants, nondisjunction of the X chromosome is elevated 30-fold [23]. In wild-type females, most X chromosomes that nondisjoin did not have any crossovers, had only a single crossover that was distal, or had a centromere-proximal crossover [27]. We analyzed X chromosomes that nondisjoined in *Blm* mutants. In 33 of 34 cases, the nondisjoined chromosomes had no crossovers; the remaining case had a single crossover in the most centromere-proximal interval (Figure 3B).

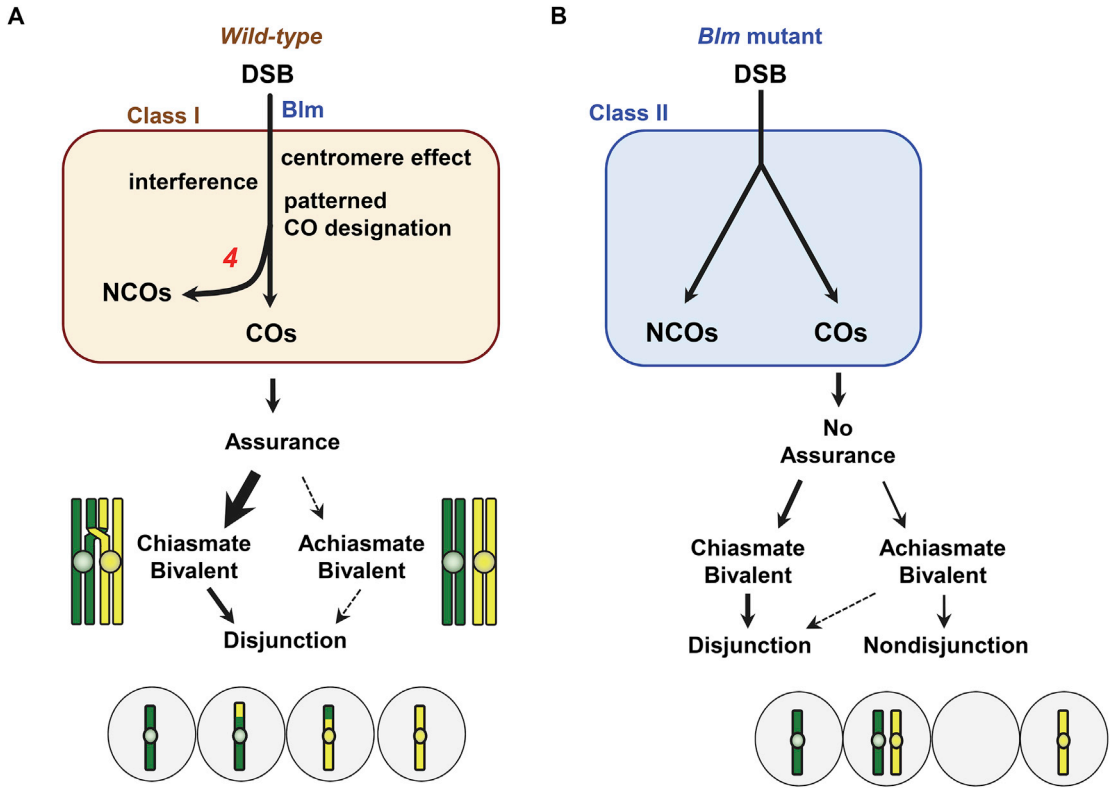

Figure 4. Crossover Patterning Promotes Proper Disjunction

(A) In wild-type flies, crossovers (COs) are produced almost exclusively by the class I pathway. Entry into this pathway requires Blm helicase activity. Interference and the centromere effect impact which intermediates will be designated to become crossovers. Repair intermediates not selected to become crossovers are repaired into noncrossovers (NCOs). On chromosome *4*, crossover patterning processes prevent all recombination intermediates from earning the crossover designation, so all are repaired as noncrossovers (indicated by red *4*). Highly regulated crossover patterning ensures that each bivalent receives at least one crossover designation, leading to crossover assurance. Assurance is not absolute in *Drosophila*, so some achiasmate bivalents remain; however, the achiasmate segregation pathway ensures accurate disjunction of these.

(B) In *Blm* mutant flies, the class I pathway is not populated, so all DSBs are repaired by the backup class II pathway. Because the class II pathway lacks crossover patterning, every DSB has a fixed probability of becoming a crossover, regardless of genomic location. Crossover assurance is absent, leading to an elevation in achiasmate bivalents. The achiasmate segregation system cannot compensate for the high number of E_0 bivalents, so nondisjunction is elevated.

Most X nondisjunction in *Blm* mutants occurs between chromosomes that did not experience a crossover. The incidence of E_0 X chromosomes was elevated in *Blm* mutants due to a combination of decreased crossover frequency and loss of assurance (Figure 3A). To separate these effects, we analyzed *Blm rec* double mutants. REC, the *Drosophila* ortholog of MCM8, is required in the class I crossover pathway [11, 28]. Crossovers are greatly reduced in *rec* single mutants but were elevated above wild-type levels in *Blm rec* double mutants [11] (Figure S3A). The reasons for this elevation are unknown, but they may be related to the poorly understood role of REC in the noncrossover pathway [28]. Despite the elevated crossover frequency, nondisjunction rates were similar in *Blm* mutants and *Blm rec* double mutants [11]. Like *Blm* single mutants, *Blm rec* double mutants exhibited a loss of interference, the centromere effect, and crossover assurance, and crossovers occurred on chromosome *4* (Figures S3B–S3D). These results argue that the elevated nondisjunction seen in *Blm* mutants is due primarily to the loss of crossover patterning.

Interference, the centromere effect, and the obligate chiasma were all described more than 80 years ago [4, 14, 24], but the mechanisms behind these phenomena remain unknown. These phenomena are entwined in the class I crossover pathway, but it is unclear whether they are generated independently within this pathway or are merely different manifestations of a single regulatory process. Mathematical modeling has suggested that an obligatory crossover is ensured by a combination of interference and other features of the class I pathway, so these processes may be inter-dependent [2, 29]. The centromere effect may be an augmentation that reinforces interference by pushing crossovers toward the middle of the arm [30]. However, since the telomere effect in *Drosophila* was far weaker than the centromere effect (Figure 1B), it seems likely that the centromere effect is an independent phenomenon that functions to prevent proximal crossovers, presumably because these can induce nondisjunction [27]. We identified only a single case of a proximal crossover in the set of nondisjoined chromosomes we analyzed, but this was a significant increase from the frequency in wild-type females (one case in ~2,900 progeny in *Blm* mutants compared to six cases from ~600,000 progeny of wild-type females [27]; p = 0.0109).

The meiotic function of *Drosophila* Blm appears to be similar to the role of *S. cerevisiae* Sgs1 in allowing recombination

intermediates to populate the class I crossover pathway [7, 8], but this is not conserved in some other species. In *Arabidopsis*, redundant Blm paralogs prevent class II crossovers, perhaps by promoting noncrossover repair, but are not required for class I crossovers [31]. The *C. elegans* ortholog, HIM-6, does have a role in making class I crossovers [32]; however, this occurs after normal crossover designation. This is not unlike *Drosophila mei-9* mutants, where crossover designation is intact but crossover formation is impaired, resulting in the few crossovers that are made having a wild-type distribution [10] (Figure S1B).

In summary, we have assessed the importance of crossover patterning in meiosis by exploiting the loss of patterning in *Drosophila* mutants lacking Blm helicase. In wild-type females, the primary meiotic recombination pathway incorporates the centromere effect and interference to promote patterned designation of which events will become crossovers (Figure 4A). Strong crossover assurance means that most homologous chromosomes have a crossover that ensures their disjunction, but the few achiasmate pairs are still segregated accurately by the achiasmate segregation system. Blm is essential for entrance into this meiosis-specific class I repair pathway; in *Blm* mutants, repair instead occurs through the class II pathway (Figure 4B). These crossovers lead to chiasmata that are competent to promote accurate disjunction. However, because crossover patterning is lost, there is an elevated frequency of chromosomes at risk for nondisjunction (primarily achiasmate chromosomes, but possibly also chromosomes with very proximal crossovers).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.10.055.

AUTHOR CONTRIBUTIONS

T.H., K.P.K., S.M., M.A.H., and A.M.W. collected data. T.H., K.P.K., M.A.H., and J.S. analyzed data. T.H., K.P.K., and J.S. wrote the manuscript. T.H. and K.P.K. contributed equally. All authors read and approved the final manuscript.

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