Genetic analysis reveals novel roles for mei-MCM proteins during meiotic recombination in *Drosophila*

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Abstract

Crossover formation as a result of meiotic recombination is vital for proper segregation of homologous chromosomes at the end of meiosis I. In most organisms, crossovers are generated through two crossover pathways: Class I and Class II. To ensure accurate crossover formation, meiosis-specific protein complexes regulate the degree in which each pathway is used. One such complex is the mei-MCM complex, which contains MCM (mini-chromosome maintenance) and MCM-like proteins REC (ortholog of Mcm8), MEI-217, and MEI-218, collectively called the mei-MCM complex. The mei-MCM complex genetically promotes Class I crossovers and inhibits Class II crossovers in *Drosophila*, but it is unclear how individual mei-MCM proteins contribute to crossover regulation. In this study, we perform genetic analyses to understand how specific regions and motifs of mei-MCMs contribute to Class I and II crossover formation and distribution. Our analyses show that the long, disordered N-terminus of MEI-218 is dispensable for crossover formation, and that REC's predicted ability to bind and hydrolyze ATP is differentially required for Class I and Class II crossover formation. Results indicate that REC's predicted ability to hydrolyze ATP, in *trans* with an additional, unknown subunit is required for promoting the formation of Class I crossovers. However, the inhibition of Class II

crossovers depends on REC's predicted abilities to both bind and hydrolyze ATP. Overall, our results suggest that REC forms multiple complexes that exhibit differential REC-dependent ATP binding and hydrolyzing requirements. These results provide genetic insight into the mechanism in which mei-MCMs, a conserved class of meiotic proteins, promote Class I crossovers and inhibit Class II crossovers.

Introduction

In order to reestablish the diploid genome upon sexual fertilization, the genome of progenitor germ cells must be successfully reduced by half through meiosis. Accurate reduction of the genome at the end of meiosis I requires crossover formation between homologous chromosomes during meiotic recombination. Meiotic recombination is initiated by the formation of multiple double-strand breaks (DSBs); the majority of meiotic DSBs are repaired as noncrossovers, while a selected subset are repaired as crossovers between homologs (reviewed in (Lake and Hawley 2012).

Two distinct types of meiotic crossovers have been described: Class I and Class II. First defined in budding yeast (De los Santos et al. 2003), Class I and Class II crossovers exist in most sexually reproducing organisms, but the relative proportions of each crossover type vary among organisms (Hollingsworth and Brill 2004). In *Drosophila*, most – if not all – crossovers are generated through the Class I pathway (Hatkevich et al. 2017), as shown through their dependence on the putative catalytic unit of the Class I meiotic resolvase MEI-9 (Radford et al. 2005; Sekelsky et al. 1995; Yildiz et al. 2002, 2004; Radford et al. 2007). Most crossovers in *Drosophila* are also dependent upon a group of MCM- or MCM-like proteins, called the mei-MCM complex (Baker and Carpenter 1972a; Grell 1978; Liu et al. 2000; Kohl, Jones, and Sekelsky 2012).

The mei-MCM complex consists of REC (the *Drosophila* ortholog of MCM8), MEI-217, and MEI-218. REC appears to be a bona fide MCM protein, based on conservation of both the N-terminal MCM domain and the C-terminal AAA+ ATPase domain, which includes Walker A and B boxes that bind and hydrolyze ATP (Figure 1A). In contrast, MEI-217 and MEI-218 are highly divergent MCM-like proteins, and together resemble one full MCM protein. MEI-217 is structurally similar to the MCM N-terminal domain, though this similarity is not detected in BLAST or conserved domain searches (Kohl, Jones, and Sekelsky 2012). The carboxy-terminus of MEI-218 has a domain related to the AAA+ ATPase domain, but key residues are not conserved, including the Walker A and B motifs that are critical for binding and hydrolyzing ATP, respectively (Iyer et al. 2004) (Figure 1B). In addition, MEI-218 has a long N-terminal extension that is poorly conserved and predicted to be disordered. The function of this region is unknown, but gene swap studies suggest that it may contribute to differences in the recombination landscape among *Drosophila* species (Brand et al. 2018). For further analysis and details regarding the evolution of the mei-MCM complex, see Supplemental Figures S1-S3.

While most crossovers are generated through the Class I pathway in wild-type *Drosophila* and are mei-MCM dependent, mutants that lack the Bloom syndrome helicase (Blm) generate only Class II crossovers based on their independence of MEI-9 and lack of patterning (*e.g.*, interference) that is associated with Class I crossovers (Hatkevich et al. 2017). Blm is an ATP-dependent 3'-5' helicase that exhibits vital anti-crossover functions in both meiotic and somatic DSB repair (reviewed in Hatkevich and Sekelsky 2017). Interestingly, mutations in *mei-MCM* and *Blm* genes genetically interact. In *Blm* mutants, crossovers are reduced by 30% but in a *Blm rec* double mutant, crossovers are significantly increased compared to wild-type (Kohl,

Jones, and Sekelsky 2012). This suggests that the mei-MCMs may function to inhibit crossovers within the Class II pathway, in addition to their role promoting crossovers in the Class I pathway.

While the mei-MCMs function as a complex, little is known about how individual mei-MCMs contribute to Class I and II crossover regulation. Here, we investigate specific features of MEI-218 and REC to understand better how these proteins contribute to meiotic recombination. We find that the N-terminus of MEI-218 is dispensable for crossover formation and general crossover distribution. Interestingly, the predicted ability for REC to bind and hydrolyze ATP are differentially required within the Class I and II crossover pathways, such that REC ATP hydrolysis activity, but not ATP binding activity, is required for Class I, MEI-9-dependent crossovers, but both ATP activities function to inhibit Class II crossovers. Our results suggest that the mei-MCMs function in multiple roles and may complex in a variety of configurations in order to properly regulate crossover formation.

Materials and Methods

Drosophila stocks. Flies were maintained on standard medium at 25°C. Some mutant alleles have been previously described, including *mei-9*^{a} (Baker and Carpenter 1972b; Yildiz et al. 2004), *mei-218*^{1} and *mei-218*^{6} (Baker and Carpenter 1972b; McKim, Dahmus, and Hawley 1996), *Blm*^{N1} and *Blm*^{D2} (McVey et al. 2007), *rec*^{1} and *rec*^{2} (Matsubayashi and Yamamoto 2003; Grell 1978). The maternal-effect lethality in *Blm*^{N1}/*Blm*^{D2} mutants was overcome by the *UAS::GAL4* rescue system previously described (Kohl, Jones, and Sekelsky 2012).

Generating mei-218 transgenic alleles. The transgenes for *mei-218*$^{\Delta N}$ and *mei-218*^{FL} were constructed by cloning cDNA for *mei-218* into *p{attBUASpW}* (AddGene). Full-length *mei-218*

included codons 1-1186; the *mei-218*$^{\Delta N}$ transgene included codons 527-1186. Transgenics were made by integrating into a phiC31 landing site in 2A on the *X* chromosome.

Generating* rec^{KA} *and* rec^{DA} *mutants. Annealed oligonucleotides were inserted into *Bbs*I-digested pU6-BbsI-chiRNA plasmid (Addgene). *rec*^{KA}: CTTCGCCGAGAAGGGATAGTAAAC; *rec*^{DA}: CTTCGTTGCAGTGCCTACAATCAG. Resulting plasmids were co-injected with repair template plasmid, consisting of synthesized gBlocks (IDT DNA) cloned into pBlueScript plasmid (sequences available on request). Injected larvae were raised to adulthood, and their male progeny were crossed to *TM3*/*TM6B* females (Bloomington Stock Center) to generate stocks, after which DNA was extracted for screening through PCR and restriction digest.

Nondisjunction assay. *X*-chromosome nondisjunction (NDJ) was assayed by mating virgin females to *y cv v f* / *T(1:Y)B*^{S} males. Each cross was set up as a single experiment with 20-50 separate vials; the progeny of each vial were counted separately. Viable nondisjunction progeny are *XXY* females with Bar eyes and *XO* males with Bar^{+} eyes and the phenotypes from *y cv v f* chromosome. Total (adjusted) represents the total with inviable exceptional progeny accounted for (*XXX* and *YO*). NDJ rates and statistical comparisons were done as in Zeng et al. 2010.

Crossover distribution assay. Crossover distribution on chromosome *2*L was scored by crossing virgin *net dpp*$^{d\text{-}ho}$ *dp b pr cn* / + female flies with mutant background of interest to *net dpp*$^{d\text{-}ho}$ *dp b pr cn* homozygous males. Each cross was set up as a single experiment with at least 25 separate vials scored. All progeny were scored for parental and recombinant phenotypes. Crossover numbers in flies are shown as cM where cM = (number of crossovers / total number of flies) * 100. Chi-squared tests with Bonferroni correction were performed for each interval. For

total cM, Fisher's Exact Test was used to compare total crossovers to total number of flies. Crossover distribution is represented as cM/Mb where Mb is length of the interval without transposable elements (TEs) because crossovers rarely occur within TEs (Miller et al. 2016).

Protein structure and alignment. Structural domains of proteins were determined by using PHYRE 2. All of the MCM regions identified correspond to the protein data bank ID #c2vl6C and the AAA ATPase domains identified correspond to protein data bank ID #d1g8pa. Alignment of the Walker A and Walker B motifs (Kohl, Jones, and Sekelsky 2012) was done using MEGA 5 and aligned with the ClustalW program. Identical and conserved residues are shaded based on groups of amino acids with similar chemical properties.

Data availability. All data necessary for confirming the conclusions in this paper are included in this article and in supplemental figures and tables. *Drosophila* stocks and plasmids described in this study are available upon request. We have uploaded Supplemental Material to Figshare.

Figure S1 illustrates distribution of Msh4, Msh5, Mcm8, Mcm9, MEI-217, and MEI-218 in Diptera. Figure S2 illustrates the structure of MEI-217 and MEI-218 in Diptera. Figure S3 shows sequence alignment of MEI-218. Figure S4 details the cross scheme of *mei-218* transgene expriments. Table S1 includes analysis of genetic interval differences between *WT* and *mei-218*^{FL}. Table S2 includes analysis of genetic interval differences between *mei-218*^{FL} and *mei-218*$^{\Delta N}$. Table S3 includes complete data set for calculating nondisjunction of *WT, rec*$^{-}$*/rec*$^{+}$, and *rec*^{DA}/+. Table S4 includes all data sets for meiotic crossovers for all genotypes discussed.

Results and Discussion

The N-terminus of MEI-218 is dispensable for crossover formation

MCMDC2 is a distantly-related member of the MCM family of proteins that is unique in that the ATPase domain is predicted to be incapable of binding or hydrolyzing ATP. Orthologs in Dipteran insects are further distinguished by having the N-terminal and ATPase-like domains encoded in separate open reading frames. The two polypeptides, MEI-217 and MEI-218 interact physically, at least in *Drosophila melanogaster*, presumably reconstituting a single MCM-like protein. MEI-218 is also distinguished by possessing an N-terminal extension of variable length in different species. *Drosophila melanogaster* MEI-218 can be divided into three distinct regions (Figure 1A): an N-terminal tail (residues 1-500), a central acidic region (residues 500-800) and the C-terminal ATPase-related region (residues 850-1116) (Brand et al. 2018; Kohl, Jones, and Sekelsky 2012). The N-terminal and middle regions are predicted to be disordered (Kohl, Jones, and Sekelsky 2012) and are poorly conserved (Figure S3). Results obtained through gene swap experiments suggest that the N-terminal tail and central region regulate crossover number and distribution within *Drosophila* species (Brand et al. 2018).

To genetically examine the function of the N-terminus of MEI-218, we compared functions of a transgene that expresses a truncated form of MEI-218 that lacks the N-terminal 526 amino acids (*mei-218*$^{\Delta N}$) to a matched full-length transgene (*mei-218*^{FL}) (Figure 2A). Due to the relatively high conservation among *Drosophila* species, the middle region of mei-218 was retained for this experiment (Figure S3). Using the *UAS*/*GAL4* system (Duffy 2002), we expressed both constructs in *mei-218* null mutants using the germline-specific *nanos* promoter and measured crossovers along five adjacent intervals that span most of *2*L and part of *2R* (Figure S4; for simplicity, we refer to this chromosomal region as *2*L.)

In wild-type females, the genetic length of *2*L is 45.8 cM (Hatkevich et al. 2017) (Figure 2B), whereas *mei-218* mutants exhibit a severe decrease in crossovers, with genetic length of 2.92 cM (Kohl, Jones, and Sekelsky 2012). Expression of *mei-218*^{FL} in *mei-218* mutants (*mei-218*^{FL}) fully rescues the crossover defect, exhibiting a genetic length of 54.1 cM. Unexpectedly, expression of *mei-218*$^{\Delta N}$ in *mei-218* mutants (*mei-218*$^{\Delta N}$) restored crossing over to the same level as *mei-218; mei-218*^{FL} (55.9 cM; *n.s.* $p = 0.61$).

Brand *et al.* (2018) expressed *Drosophila mauritiana* MEI-217 and MEI-218 in *Drosophila melanogaster* and found that crossovers were increased in proximal and distal regions, resulting in an overall change in crossover distribution. To determine whether the N-terminus of *Drosophila melanogaster* MEI-218 functions in regulating crossover distribution, we examined crossover distribution in *mei-218; mei-218*^{FL} and *mei-218; mei-218*$^{\Delta N}$ (Figure 2C). Overall, crossover distributions are similar, with both genotypes exhibiting a strong inhibition of crossovers near the centromere (referred to as the centromere effect; Beadle 1932) and the majority of the crossovers placed in the medial-distal regions.

We conclude that the N-terminal tail of MEI-218 is dispensable for both crossover formation and overall distribution on chromosome *2*L. Recently, Brand et al. suggest that the variation among *Drosophila* MEI-218 N-terminal and middle-acidic regions, which appear to be under positive selection, account for the differences in recombination rate and patterning between *Drosophila melanogaster* and *Drosophila mauritiana*. However, our results suggest that the N-terminal region of *D. melanogaster* MEI-218 is not required to establish the recombination landscape in *D. melanogaster*.

The reasons why the MCM domains have been separated into MEI-217 and MEI-218 and why MEI-218 has an N-terminal extension are unknown, but this structure has been maintained

for more than 250 million years of Dipteran evolution. Interestingly, MEI-218 is expressed moderately highly in testes (Thurmond et al. 2018, FB2018_05) even though males do not experience meiotic recombination. The predominant or exclusive transcript in males does not encode MEI-217 (Thurmond et al. 2018, FB2018_05), the seemingly obligate partner for MEI-218. Further, males that lack *mei-218* are viable, fertile, and do not exhibit nondisjunction (Baker and Carpenter 1972b; McKim, Dahmus, and Hawley 1996). For these reasons, we speculate that MEI-218 functions independent of MEI-217 in *Drosophila* male testes development, providing a reason as to why its structure has been evolutionarily maintained.

REC ATPase activity is required for crossover formation

Of the three known mei-MCM subunits, only REC harbors well-conserved Walker A and B motifs, suggesting that REC has ATP binding and hydrolysis activity (Kohl, Jones, and Sekelsky 2012). It is unknown whether the mei-MCM complex utilizes REC's putative ATPase activity for its function *in vivo*. To test this, we used CRIPSR/Cas9 to introduce into *rec* mutations predicted to disrupt Walker A and B motif functions (Figure 3A). The Walker A mutation (rec^{KA}) results in substitution of a conserved lysine residue with alanine; the cognate mutation in other AAA+ ATPases, including MCMs, prevents binding of ATP (Bell and Botchan 2013). The Walker B mutation (rec^{DA}) results in substitution of a conserved aspartic acid with alanine; in MCMs and other AAA+ ATPases, the cognate mutation results in the inability to hydrolyze ATP (Bochman, Bell, and Schwacha 2008).

We assayed crossover frequency along *2*L in rec^{KA} and rec^{DA} mutants (Figure 3B). Surprisingly rec^{KA} ATP binding mutants exhibit a genetic length of 44.9 cM, which is not significantly different from wild-type (p = 0.4016), suggesting that ATP binding by REC is not

required for crossover formation. Conversely, there is a severe reduction in crossovers in rec^{DA} mutants, with a genetic length of 1.6 cM (***$p < 0.0001$), indicating that REC's ability to hydrolyze ATP is required for crossover formation.

Because the genetic length of rec^{DA} is significantly lower than *rec* null mutants (Figure 3B, $p < 0.0001$), we hypothesized that rec^{DA} is an antimorphic mutation. To test this, we examined crossover levels and *X* chromosome nondisjunction (NDJ) in rec^{DA}/rec^{+} (Figure 3B and 3C, respectively). The genetic length of *2*L in $rec^{DA/+}$ is slightly lower than wild-type, but not significantly different (43.9 cM and 45.8 cM, respectively; $p = 0.35$). For *X*-NDJ, both wild-type and rec^{-}/rec^{+} mutants exhibit rates below 0.5%, while rec^{DA}/rec^{+} mutants exhibit a significant increase to 1.4% NDJ ($p < 0.0001$). These data support the conclusion that rec^{DA} is weakly antimorphic and suggests that rec^{DA} produces an inactive mei-MCM complex that is antagonistic to the wild-type complex. In light of these interpretations, we propose that the mei-MCM complex binds to recombination sites independent of REC binding to ATP, and that REC-dependent ATP hydrolysis is required for the removal of the mei-MCM complex from these sites.

We conclude that REC's putative ATPase activity is needed for crossover formation. Specifically, REC's ability to hydrolyze ATP is required for crossover formation, but REC's binding capability is apparently dispensable. The disparate requirements for REC's ATP binding and hydrolysis are similar to studies of other ATPase-dependent complexes. Rad51 paralogs, which form multi-protein complexes and contain Walker A and B motifs, are proposed to exhibit ATPase activity in *trans* between adjacent subunits, with each subunit exhibiting differential ATP binding and hydrolysis requirements for ATPase activity within the complex (Wiese et al. 2006; Wu et al. 2004, 2005). Additionally, in canonical MCM proteins, mutations within the

different subunits' Walker A and B motifs have varying effects on ATPase activity (Gómez, Catlett, and Forsburg 2002). Because neither MEI-217 nor MEI-218 possess a conserved ATPase domain (Figure 1B) (Kohl, Jones, and Sekelsky 2012), we propose that ATPase activity of the mei-MCM complex requires REC for ATP hydrolysis and an unknown mei-MCM protein for ATP binding (Figure 3D). Further studies are needed to uncover this hypothesized novel mei-MCM.

REC-dependent ATP hydrolysis is required for MEI-9-dependent crossovers

To gain insight into the crossover pathways that are used in *rec*^{KA} and *rec*^{DA} mutants, we examined whether these crossovers require the Class I nuclease. In *Drosophila,* the catalytic subunit of the putative Class I meiosis-specific endonuclease is MEI-9 (Radford et al. 2005; Sekelsky et al. 1995; Yildiz et al. 2002, 2004; Radford et al. 2007; Hatkevich et al. 2017). The *2*L genetic length within a *mei-9* mutant is 2.75 cM (Figure 4), demonstrating that at least 90% of crossovers are dependent upon MEI-9. However, the genetic length in *rec* null mutants is barely significantly lower than *mei-9; rec* double mutants, indicating that in the absence of REC, the resulting crossovers are likely independent of MEI-9 (p = 0.047). Similarly, it has been shown previously that *mei-218 mei-9* double mutants do not experience differences in crossovers compared to a *mei-9* single (Sekelsky et al. 1995), indicating that crossovers generated in the absence of the mei-MCM complex are MEI-9 independent.

Because *rec*^{KA} mutants exhibit the same distribution and number of crossovers as wild-type (Figure 3B), we hypothesized that *rec*^{KA} crossovers are dependent on MEI-9. To test this, we examined genetic length across *2*L in *mei-9; rec*^{KA} double mutants (Figure 4). Mutants for *mei-9; rec*^{KA} exhibit a genetic length of 2.72 cM, which is significantly decreased compared

to the *rec*^{KA} single mutant ($p < 0.0001$), but not significantly different from *mei-9* single mutants ($p = 0.94$), showing that crossovers in *rec*^{KA} are dependent upon MEI-9 nuclease. In contrast, due to the dominant negative nature of *rec*^{DA}, we predicted that crossovers in *rec*^{DA} will be independent of MEI-9, similar to crossovers generated in *rec* null mutants. We observe that *mei-9; rec*^{DA} double mutants exhibit a genetic length of 1.1 cM, which is significantly lower than *mei-9* single mutants ($p < 0.001$). Importantly, crossing over in the *mei-9; rec*^{DA} double mutant is not significantly different than in *rec*^{DA} single mutants ($p = 0.23$), demonstrating that crossovers in *rec*^{DA} are independent of MEI-9 resolution (Figure 4).

From these data, we conclude that MEI-9 generates the crossovers in *rec*^{KA} mutants, whereas mitotic nucleases generate the residual crossovers in *rec*^{DA} mutants. These data show that *rec*^{KA} appears to function as wild-type in the Class I pathway, while Class I crossovers are lost in *rec* null and *rec*^{DA} mutants. We suggest that the REC's ability to hydrolyze, but not bind, ATP is required for the formation of Class I crossovers.

REC ATP binding and hydrolysis are required to prevent Class II crossovers

In wild-type *Drosophila*, most or all crossovers are generated through the Class I pathway (Hatkevich et al. 2017), and these crossovers are dependent upon the mei-MCM complex (Kohl, Jones, and Sekelsky 2012). However, in *Blm* mutants, crossovers are generated exclusively through the Class II pathway (Hatkevich et al. 2017). In *Drosophila Blm* mutants, meiotic crossovers are decreased by 30%, suggesting that the Class II pathway is less efficient at generating crossovers than the Class I pathway, even though what may be the primary anti-crossover protein, Blm helicase, is absent. It has previously been shown that loss of Blm suppresses the high nondisjunction of *mei-218* and *rec* mutants (Kohl, Jones, and Sekelsky

2012). However, in *Blm rec* double mutants, crossovers are increased significantly compared to *Blm* single mutants (Kohl, Jones, and Sekelsky 2012), suggesting that REC and/or the mei-MCM complex has an anti-crossover role in *Blm* mutants (and therefore in the Class II crossover pathway).

To further understand the role of REC in the Class II pathway, we investigated whether REC's predicted ATP binding or hydrolysis function is required for its Class II anti-crossover function. To do this, we measured the crossovers across *2*L in rec^{KA} and rec^{DA} in the background of *Blm* mutants. If REC ATP binding or hydrolysis is required for an anti-crossover role in Class II, then the genetic length of *Blm* rec^{KA} or *Blm* rec^{DA} double mutants will be similar to that of *Blm rec* double mutants. Conversely, if REC ATP binding or hydrolysis is not required, then double mutants will exhibit genetic lengths similar to that of *Blm* single mutants.

Interestingly, *Blm* rec^{KA} mutants exhibit a genetic length of 43.3 cM, which is not significantly different than *Blm rec* mutants but significantly higher than *Blm* single mutants (p = 0.10 and $p < 0.0001$ Figure 5A). Similarly, *Blm* rec^{DA} double mutants have a recombination rate of 53.4 cM, which not significantly different from *Blm rec* double mutants, but significantly higher than *Blm* single mutants ($p = 0.52$ $p < 0.0001$). These results demonstrate that REC's predicted abilities to bind and hydrolyze ATP are required for the inhibition of crossovers at REC-associated Class II recombination sites. Therefore, it appears that REC forms different complexes within the Class II pathway and Class I pathway. It is unknown if this Class II REC-associated complex requires the additional mei-MCMs, and additional genetic studies will be valuable to discern this.

Overall, the mei-MCMs are a family of diverged proteins that help to establish the recombination landscape in *Drosophila melanogaster* by promoting Class I crossovers and

inhibiting Class II crossovers. Results obtained in this study have further elucidated meiotic recombination roles for two mei-MCMs, MEI-218 and REC. While the N-terminus of MEI-218 is dispensable for crossover formation (Figure 2), REC's predicted ability to bind and hydrolyze ATP exhibit differential requirements for regulating Class I and Class II crossover formation. REC-dependent ATP hydrolysis, but not ATP binding, is required for promoting the formation Class I, MEI-9 dependent crossovers (Figures 3 and 4). The weakly antimorphic phenotype of *rec*^{DA} demonstrates that an impaired REC Walker B mutant renders a poisonous complex – a complex in which we propose cannot be released from recombination sites. Conversely, both the ability for REC to bind and hydrolyze ATP are required for REC's Class II anti-crossover role, suggesting that REC forms different complexes to execute its pro- and anti-crossover functions. Biochemical and cytological studies are needed to support or refute these hypotheses.

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Figure Legends

Figure 1. MCM protein structure and alignments. (A) Structural domains of *Drosophila melanogaster* REC, MEI-217, MEI-218 and *Mus musculus* MCMDC2. Structural domains identified using PHYRE 2 (Kohl, Jones, and Sekelsky 2012). "MCM domain" corresponds to

protein data bank ID #c2vl6C and the AAA ATPase domains identified correspond to protein data bank ID #d1g8pa. The X on *Dm* MEI-218 and *Mm* MCMDC2 represents predicted inactive AAA ATPase domains. (B) Consensus sequence for Walker A motif (Walker et al. 1982), and consensus sequence for Walker B motif (Forsburg 2004). Identical or conserved amino acids are denoted with black background. Arrows denote the conserved catalytic residues.

Figure 2. The role of MEI-218 N-terminus in crossover formation and distribution. (A) Schematic of transgenes for full length *mei-218* and N-terminal deleted *mei-218*, in which the first 526 amino acids are absent. (B) Map units of WT (Hatkevich et al. 2017), *mei-218* (Kohl, Jones, and Sekelsky 2012), $mei\text{-}218^{FL}$ and $mei\text{-}218^{\Delta N}$. Map units represented as centimorgans (cM). Error bars indicate 95% confidence intervals. *n.s.* = not significant (p = 0.61). (C) Crossover distribution (solid lines) of $mei\text{-}218^{FL}$ and $mei\text{-}218^{\Delta N}$ represented as cM/Mb. Mb is measured distance of defined interval, excluding centromere, pericentromeric heterochromatin and transposable elements. Dotted lines represent mean crossover density across *2*L. Refer to tables S1and S3 for complete data sets.

Figure 3. REC ATPase binding and hydrolysis requirements for crossover formation. (A) Schematic representation of the mutated residues in rec^{KA} and rec^{DA}. (B) Map units of *WT* (Hatkevich et al. 2017), rec^{1}/rec^{2}, rec^{KA}, and rec^{DA}, rec^{DA}/rec^{+}. Map units represented as centimorgans (cM). Error bars show 95% confidence intervals. (C) Percent nondisjunction of *WT*, rec^{1}/rec^{+}, and rec^{DA}/rec^{+}. (D) Model of possible complex depicting the functional Walker B motif of REC protein interacting with a Walker A motif on a potential partner. * $p < 0.05$; ***$p < 0.0001$. Refer to tables S2 and S3 for complete data sets.

Figure 4. MEI-9-dependent crossovers in *rec^{KA}* and *rec^{DA}* mutants. Map units of *WT* (Hatkevich et al. 2017), *rec, mei-9, mei-9;rec, rec^{KA}, mei-9;rec^{KA}, rec^{DA},* and *mei-9;rec^{DA}*. Map units represented as centimorgans (cM). Error bars show 95% confidence intervals. * $p < 0.05$ ** $p < 0.001$ ***$p < 0.0001$; (*mei-9* vs *mei-9; rec^{KA}* $p = 0.94$) (*rec^{DA}* vs *mei-9; rec^{DA}* $p = 0.23$). Refer to table S3 for complete data set.

Figure 5. Requirements of REC ATPase activity in Blm function. Map units of *WT* (Hatkevich et al. 2017), *Blm* (Kohl, Jones, and Sekelsky 2012) , *rec, Blm rec* (Kohl, Jones, and Sekelsky 2012), *Blm rec^{KA},* and *Blm rec^{DA}*. Map units represented as centimorgans (cM). Error bars show 95% confidence intervals. Refer to table S3 for complete data set. *** $p < 0.0001$. (*Blm rec* vs *Blm rec^{KA}* $p = 0.10$) (*Blm rec* vs *Blm rec^{DA}* $p = 0.52$).

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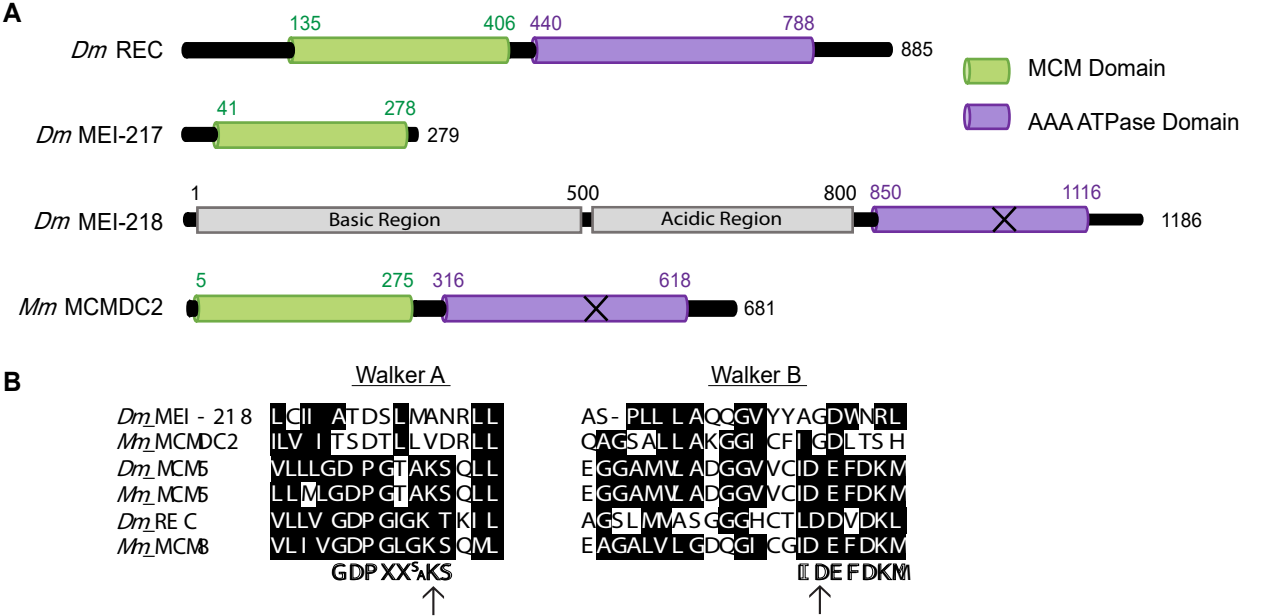
Author Summary

Crossover formation between homologs is essential for accurate segregation at the end of meiosis I. Crossovers are typically formed through two pathways: Class I and Class II. The mei-MCMs are a class of proteins that promote Class I crossover formation and prohibit Class II crossovers in Drosophila. Although the mei-MCMs are conserved, little is known about their function. Here, we investigate the roles of two mei-MCMs, REC and MEI-218, in Class I and Class II crossover formation in Drosophila. From results in this study, we generate novel, testable hypotheses to further elucidate the meiotic function of the mei-MCMs.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Dm REC
135
406
440
788
885
Dm MEI-217
41
278
279
Dm MEI-218
1
500
800
850
1116
1186
Basic Region
Acidic Region
Mm MCMDC2
5
275
316
618
681
MCM Domain
AAA ATPase Domain
B
Walker A
Walker B
Dm MEI-218
Mm MCMDC2
Dm MCM5
Mm MCM5
Dm REC
Mm MCM8
LCII ATDSLMANRLL
ILV I TSDTLLVDRLL
VLLLGD PGTAKSQLL
LLMLGDPGTAKSQLL
VLLV GDPGIGKTKIL
VLI VGDPGLGKSQML
GDPXXSAKS
AS-PLL LAQQGVYYAGDVNRL
QAGSALLAKGGI CFI GDLTSH
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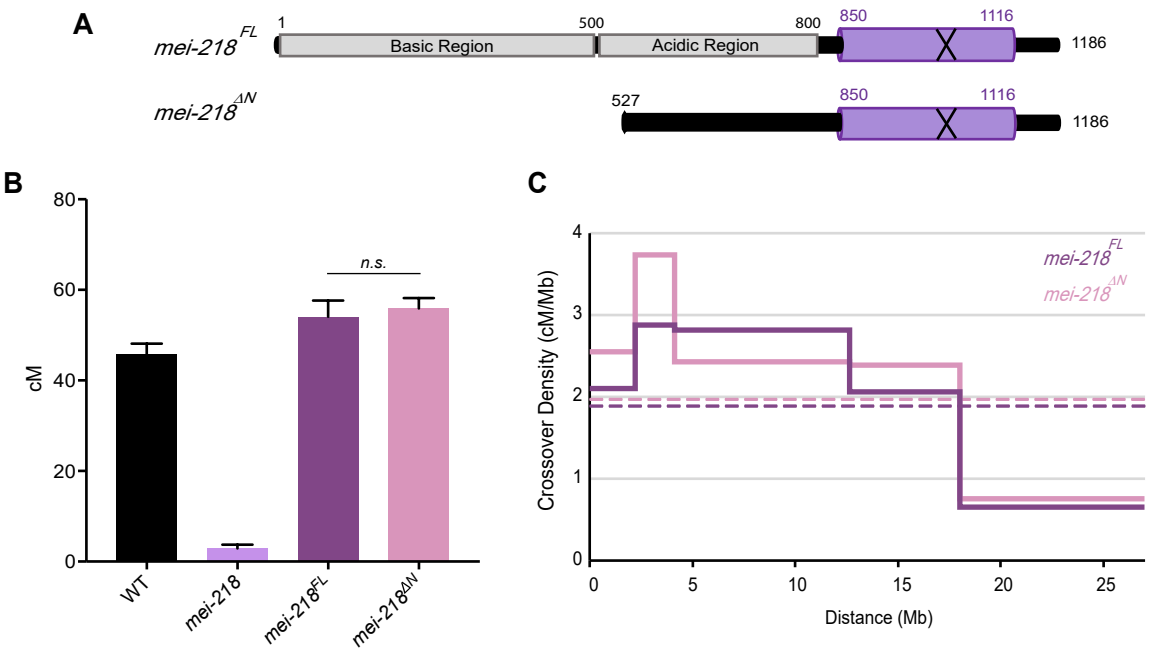
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mei-218FL
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800
850
1116
1186
Basic Region
Acidic Region
mei-218ΔN
527
850
1116
1186
B
cM
80
60
40
20
0
n.s.
WT
mei-218
mei-218FL
mei-218ΔN
C
Crossover Density (cM/Mb)
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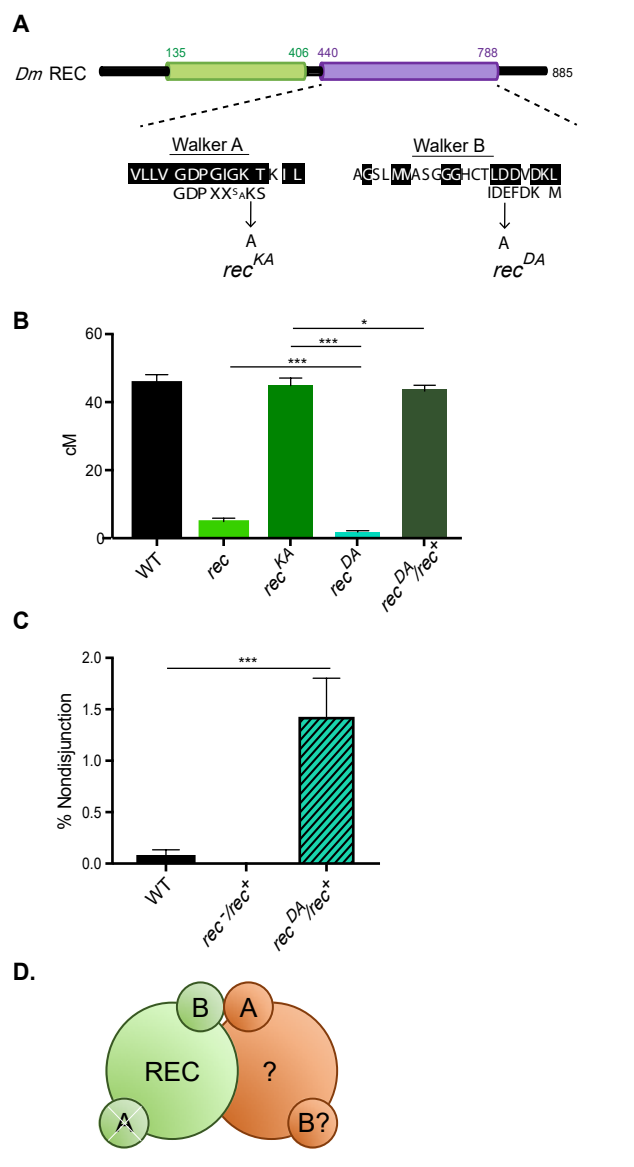
3
2
1
0
mei-218FL
mei-218ΔN
0
5
10
15
20
25
Distance (Mb)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406
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Walker A
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GDP XXSAKS
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recKA
Walker B
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IDEFDK M
A
recDA
B
cM
60
40
20
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WT
rec
recKA
recDA
recDA/rec+
C
% Nondisjunction
2.0
1.5
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0.0
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WT
rec-/rec+
recDA/rec+
D.
REC
B
A
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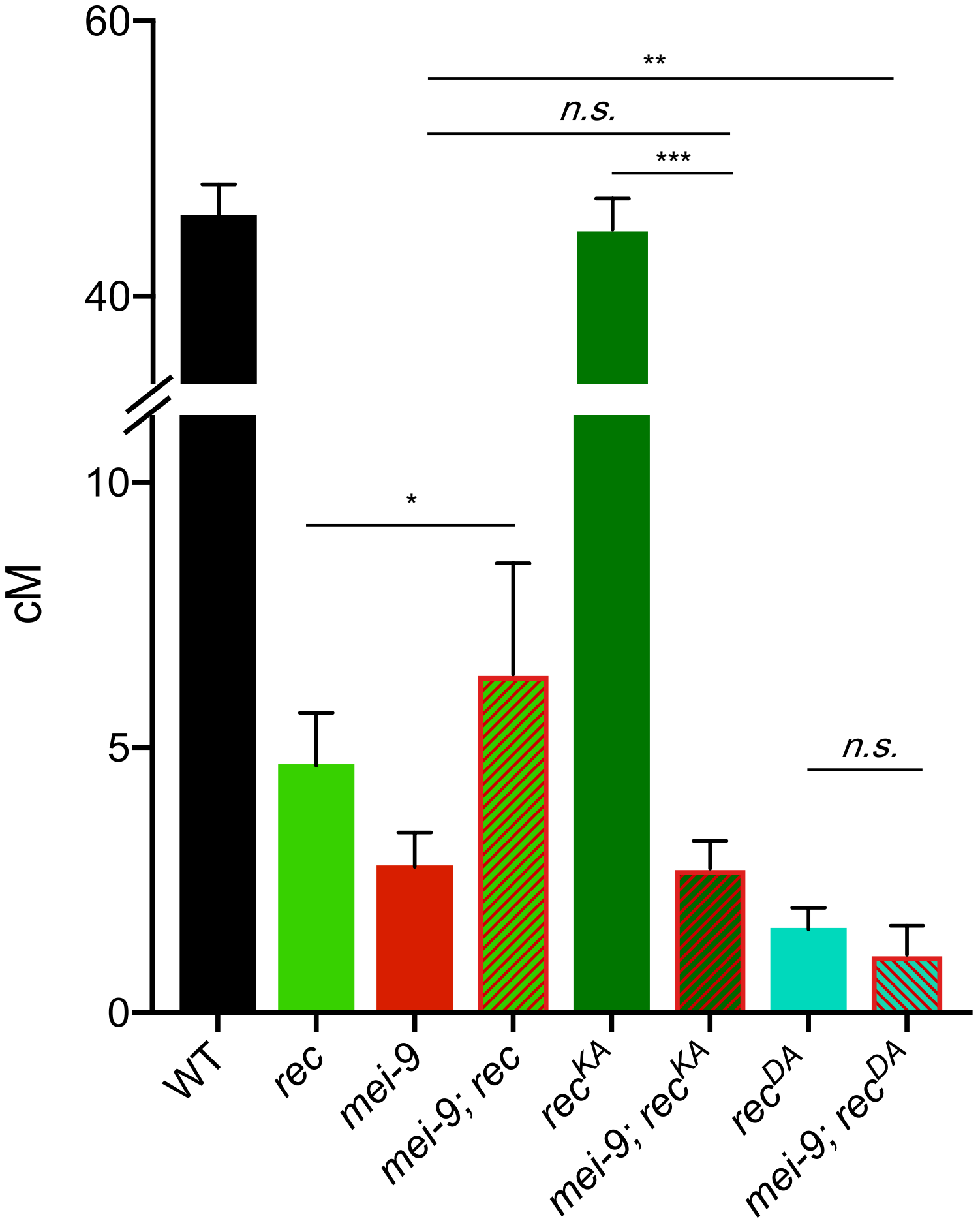
B?
A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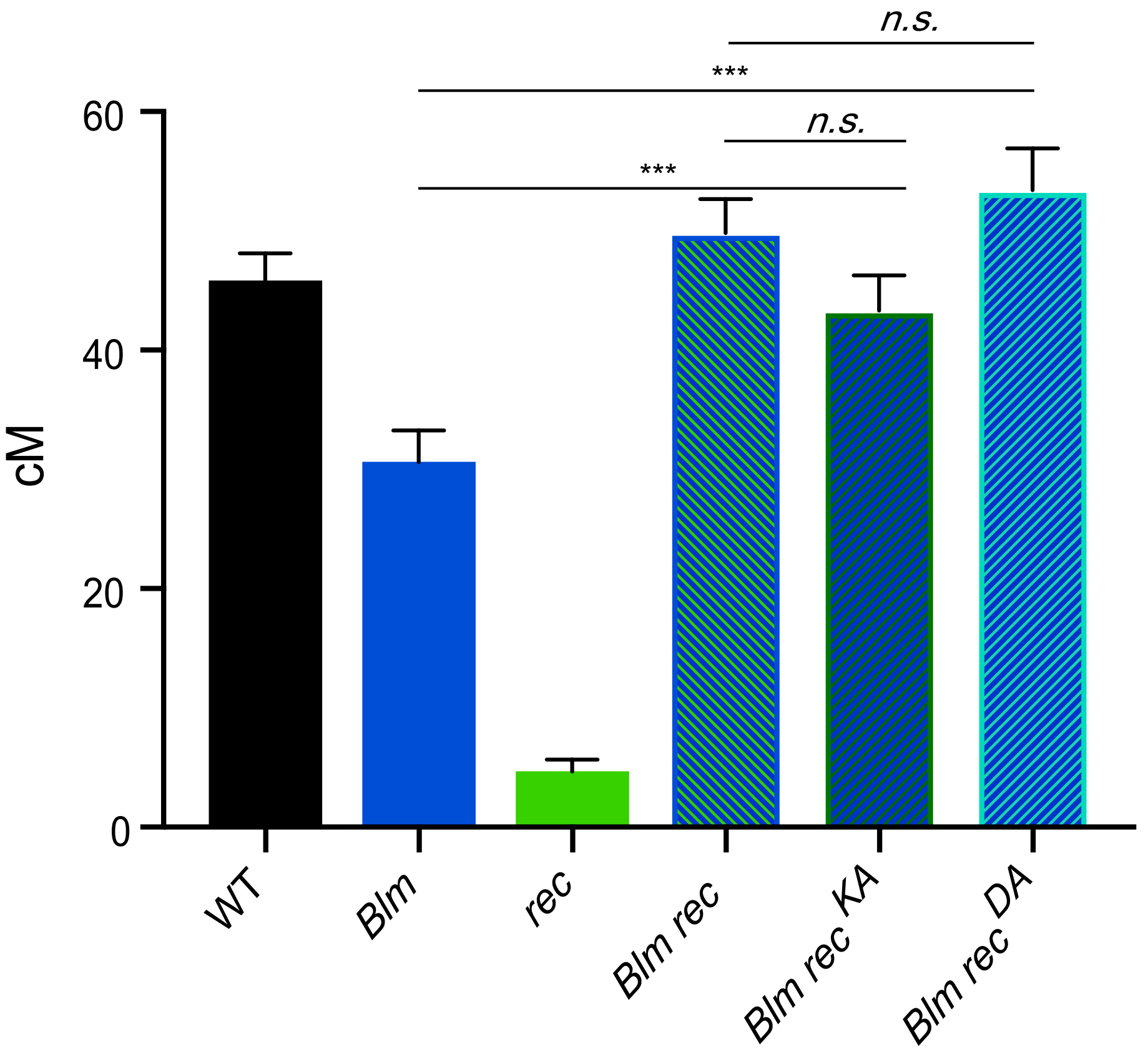
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cM
WT
Blm
rec
Blm rec
Blm rec KA
Blm rec DA