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Evolution of a MCM complex in flies promoting meiotic crossovers by blocking BLM helicase

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Abstract

Generation of meiotic crossovers in many eukaryotes requires the elimination of anti-crossover activities by utilizing the Msh4–Msh5 heterodimer to block helicases. Msh4 and Msh5 have been lost from the flies *Drosophila* and *Glossina* but we identified a complex of mini-chromosome maintenance (MCM) proteins that functionally replace Msh4–Msh5. REC, an ortholog of MCM8 that evolved under strong positive selection in flies, interacts with MEI-217 and MEI-218, which arose from a previously undescribed metazoan-specific MCM protein. Meiotic crossovers are reduced in *Drosophila rec*, *mei-217*, and *mei-218* mutants; however, removal of the Bloom syndrome helicase ortholog restores crossovers. Thus, MCMs were co-opted into a novel complex that replaces the meiotic pro-crossover function of Msh4–Msh5 in flies.

Crossovers (COs) between homologous chromosomes can be beneficial or detrimental, depending on their context (1). Meiotic COs increase genetic diversity and promote accurate chromosome segregation, whereas mitotic COs can lead to loss of heterozygosity, potentially triggering tumorigenesis. Mitotic COs are prevented by “anti-CO” proteins. A key anti-CO protein is the Bloom syndrome helicase BLM, which generates non-crossover products by unwinding recombination intermediates that might otherwise be processed into COs (2). In meiosis, CO formation is encouraged through inhibition of anti-CO proteins. The budding yeast Msh4–Msh5 heterodimer antagonizes the BLM ortholog Sgs1 (3). Msh4 and Msh5 are found in all metazoans for which sequence is available except *Drosophila* species and their fellow Schizophoran the tsetse fly *Glossina morsitans* (Figs. S1 and S2). The lack of recognizable orthologs of these proteins suggests that these species evolved another protein or complex to block the anti-CO activity of BLM.

Like *S. cerevisiae Msh4* and *Msh5* mutants, the only defects in *Drosophila rec, mei-217* and *mei-218* mutants are in meiotic recombination (4–9). REC is orthologous to MCM8 (6); MCMs have properties reminiscent of Msh4–Msh5. MCM2-7, which are essential for replication in eukaryotes, form a heterohexamer that encircles DNA (10). Similarly, Msh4–

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Msh5 is thought to encircle recombination intermediates (11). In both cases, this activity is regulated by ATP binding and hydrolysis (10, 11).

MEI-217 initially appeared to be novel, as BLAST searches fail to identify homologs outside Dipterans and searches of the Conserved Domain Database (CDD) (12) do not detect any domains. BLAST searches with MEI-218 identify a single putative ortholog in metazoans (Figs. S1 and S2). A CDD search returns a hit to the MCM domain in the C-terminus of MEI-218, but the score is low and the match covers only a third of the domain (Fig. S3). To verify the presence of this domain we conducted structure-based searches with PHYRE (Fig. 1A, (13)). This analysis reveals that the C-terminus of MEI-218 has a similar structure to the AAA ATPase domain of MCMs (Fig. S3b). Canonical MCMs have both an N-terminal MCM domain and a C-terminal ATPase domain. The N-terminal domain is present in vertebrate MEI-218 but not in *Drosophila* MEI-218. However, PHYRE with MEI-217 shows that its predicted structure is similar to the MCM N-terminal domain. Since MEI-217 and MEI-218 are encoded by overlapping open reading frames on the same transcript (7), we infer they evolved from an MCM-like protein represented by a single polypeptide in other metazoans.

The shared phenotypes and MCM domains suggest that REC, MEI-217, and MEI-218 function together in meiotic recombination. To distinguish them from the replicative MCMs, we hereafter call REC, MEI-217, and MEI-218 “mei-MCMs”. Since MCM2-7 function as a heterohexamer, we investigated whether the mei-MCMs form a complex. MEI-217 interacts strongly with both the C-terminal third of MEI-218 and REC (Figs. 1B and 1C), suggesting that the mei-MCMs form a complex. This complex likely also contains one or more replicative MCMs. A meiosis-specific mutation in *Mcm5* causes the same phenotypes as *mei-MCM* mutants (14), making MCM5 a strong candidate to be a component of the complex.

Noting the genetic and biochemical similarities between mei-MCMs and Msh4–Msh5, we hypothesized that the mei-MCMs antagonize DmBLM in lieu of Msh4–Msh5. This hypothesis predicts that removing DmBLM should compensate for *mei-MCM* mutations; in budding yeast the CO defect in *msh4* mutants is suppressed by removing Sgs1 (3). Few COs are made in *rec* and *mei-218* single mutants, resulting in high nondisjunction (NDJ) of meiotic chromosomes (Fig. 2A). In contrast, mutations in *mus309*, which encodes DmBLM, cause only a mild reduction in COs and correspondingly low levels of NDJ. Strikingly, *mus309* mutations suppress the high NDJ phenotype of *rec* and *mei-218* mutants (Fig. 2A). Furthermore, the low CO rate in *rec* mutants returns to an approximately wild-type rate in *mus309 rec* double mutants (Figs. 2B and S4, tables S1–S4), indicating that mei-MCMs are not essential for generating meiotic COs if DmBLM is absent, and supporting our hypothesis that mei-MCMs oppose the known anti-CO activities of DmBLM (15, 16).

mei-MCMs appear to functionally replace Msh4–Msh5 in Schizophora, and presumably evolved to do so in response to natural selection. Several evolutionary scenarios could lead to this result (Fig. S5), but most predict that there would be evidence of adaptive divergence of *mei-MCM* genes in Schizophora. REC was previously noted to be highly diverged in *Drosophila* (6, 17); we found that *Glossina* MCM8/REC is similarly divergent (Fig. 3). The presence or absence of MCM8 correlates with that of its functional partner MCM9 throughout eukaryotes, except in *Drosophila* and *Glossina*, which retained MCM8/REC while losing MCM9 (Fig. S1 and S2). The loss of MCM9 suggests that MCM8 evolved a novel function in an ancestor to Schizophora.

Divergence in *rec* and loss of MCM9 occurred after the split between mosquitoes and higher flies 200–250 million years ago (MYA), but prior to the emergence of the Schizophora 65

MYA. To test whether patterns of sequence evolution were consistent with positive selection leading to the divergence of *rec,* we estimated the ratio between the rate of base pair substitutions at non-synonymous sites (dN) and the rate at synonymous sites (dS) among Dipterans in *MCM8/rec*. We compared 15 evolutionary models, ranging from conservation of dN/dS ratios across all taxa surveyed to allowing free evolution of dN/dS ratios along all branches, and including models testing specific hypotheses about the evolution of *rec* along different branches of the insect phylogeny. The best fitting model ($P = 0.0002$ vs. next best model) supports the hypothesis that rapid protein coding divergence was driven by positive selection prior to the split of tsetse flies from fruit flies (Figs. 3 and S6, table S5). Thus we infer that natural selection likely drove the repurposing of REC into its new role as an antagonist of DmBLM. Recent evolution of *rec* shows much lower levels of non-synonymous changes, suggesting subsequent functional constraint (Fig. S6). MEI-217 and MEI-218 have also diverged substantially from the ancestral MCM structure: they split into two polypeptides and MEI-218 acquired an N-terminal extension (Figs. S7 and S8).

Our data show that flies evolved a novel MCM complex to antagonize anti-CO functions of BLM during meiosis, a role held by Msh4–Msh5 in other organisms. Although we do not know what evolutionary forces ultimately drove the loss of Msh4–Msh5 and the repurposing of mei-MCMs, it is tempting to speculate that these forces also led to another fundamental meiotic difference in *Drosophila* and *Glossina* compared to mosquitoes – the absence of recombination in males, which was first noted in *Drosophila* by Morgan 100 years ago (Fig. S5, (18)). Resolving the conundrum of why the mei-MCMs supplanted Msh4–Msh5 will require a deeper understanding of both the evolutionary origins of the mei-MCMs and the functional differences between mei-MCMs and Msh4–Msh5.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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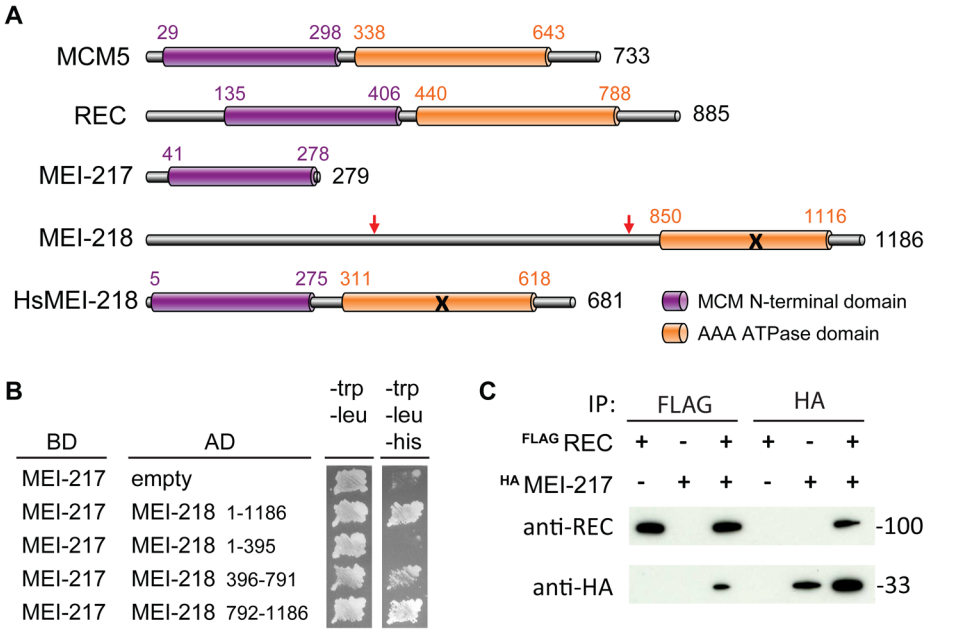

Fig. 1.

(**A**) Structural domains identified through PHYRE. “MCM N-terminal domain” corresponds to Protein Data Bank fold 3f9v and “AAA ATPase domain” to fold ID 3f8t. The “x” on MEI-218 symbolizes changes in the ATP binding and hydrolysis motifs predicted to abolish ATPase activity (Fig. S3B). Red arrows on MEI-218 indicate segments used in yeast two-hybrid analysis. (**B**) Yeast two-hybrid interactions between MEI-217 and MEI-218. Cells expressing the indicated fusions to the GAL4 DNA binding domain (BD) or activating domain (AD) were streaked onto selective media. Growth on – trp – leu – his indicates an interaction. (**C**) Co-immunoprecipitation of REC and MEI-217. Epitope-tagged mei-MCMs were co-expressed in insect cells, immunoprecipitated with anti-tag antibodies, blotted, and probed with antibodies to REC and to the HA tag (19).

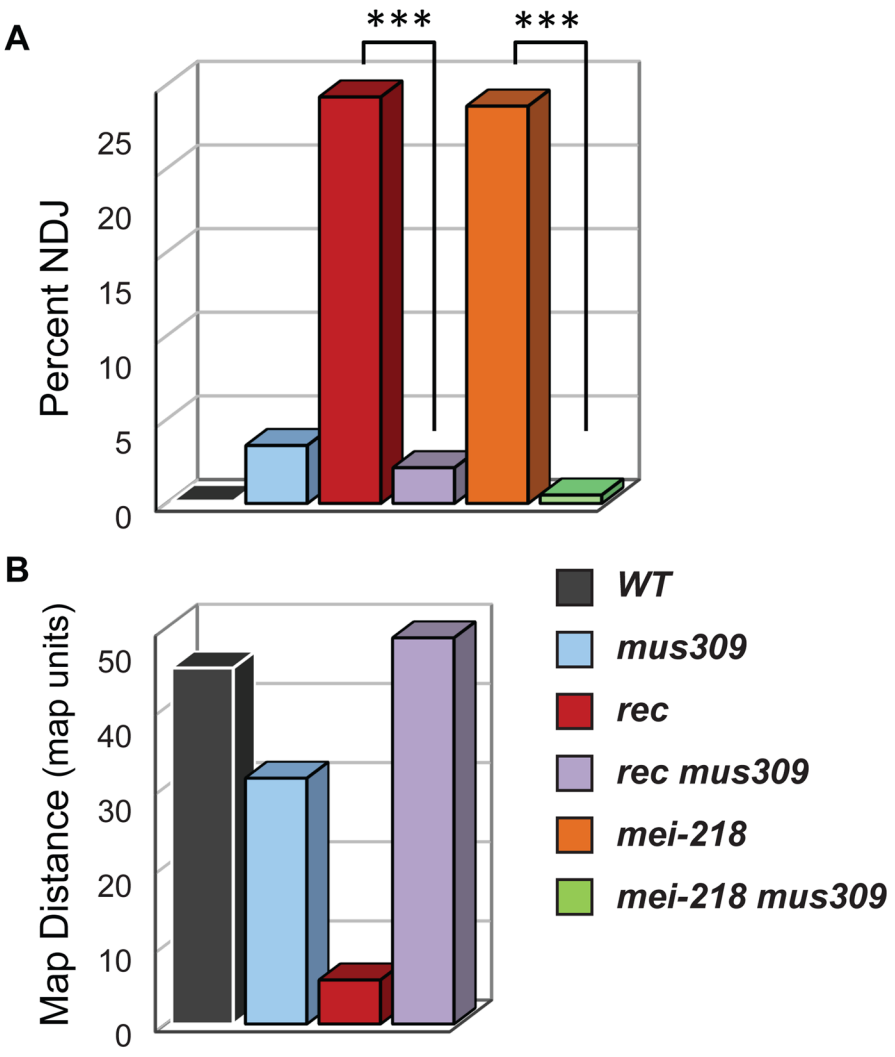

Fig. 2.

(**A**) X chromosome non-disjunction (NDJ) across more than 1500 individuals for each genotype except *mei-218; mus309* (n=383). ***, $P < 0.0001$. (**B**) This graph shows the summed map distance in map units (m. u., equivalent to centiMorgans) across five intervals spanning ~20% of the genome for over 1000 individuals for each genotype (19). $P < 0.0001$ for all comparisons except *WT* versus *mus309 rec*, $P = 0.0674$.

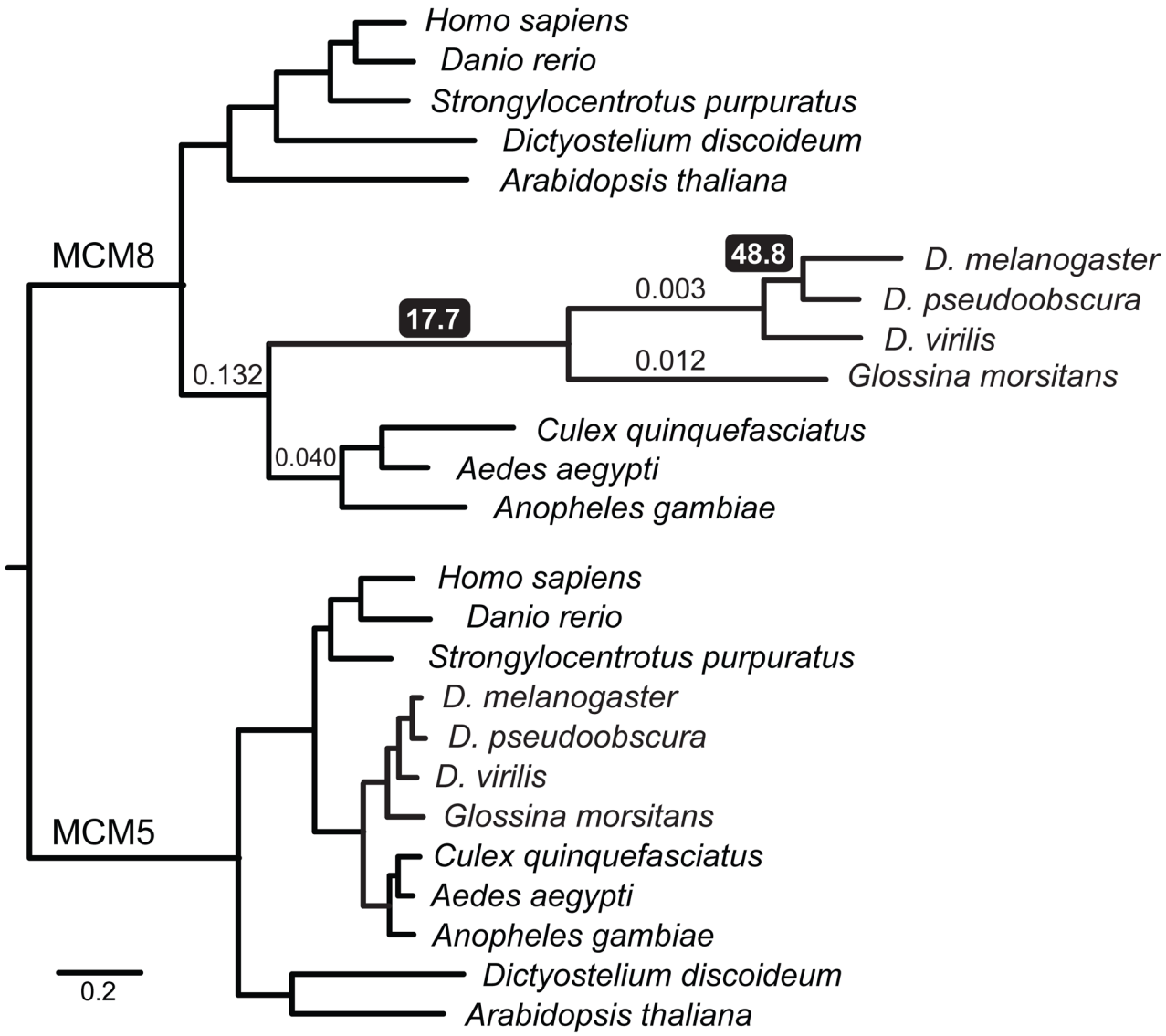

Fig. 3.

Maximum-likelihood tree from an alignment of the conserved MCM domains of MCM8/REC and MCM5 from diverse taxa. Branch lengths indicate the number of substitutions per site (see scale). Numbers above branches show dN/dS estimates for selected branches; those with black background highlight branches with dN/dS estimates greater than one, suggesting positive selection. See fig. S6 for more description and additional dN/dS estimates.