Perspective

Break to Make a Connection

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Meiosis and the Regulation of Double-Strand Break Formation

Meiosis is the cell division program utilized by most sexually reproducing organisms as a strategy to produce haploid gametes (i.e., sperm and eggs) from diploid parental cells. As suggested by its name, which stems from the Greek word meaning "to diminish or reduce", meiosis reduces the chromosome number by half. This is accomplished by following a single round of DNA replication with two consecutive rounds of cell division (meiosis I and meiosis II). At meiosis I, homologous chromosomes segregate away from each other (reductional division) and at meiosis II, sister chromatids segregate to opposite poles of the spindle (equational division). During prophase of meiosis I, chromosomes undergo a series of unique and well-orchestrated steps that promote accurate segregation. These steps include the formation of programmed DNA double-strand breaks (DSBs), homologous chromosome pairing, and synapsis. A subset of DSBs are repaired via recombination between homologous chromosomes such that there is a reciprocal exchange of genetic material between the homologs resulting in crossover formation. These crossover events, underpinned by flanking sister chromatid cohesion, generate physical attachments between the homologs (chiasmata), which are important for their proper alignment at the metaphase I plate. Either impaired DSB formation or a failure to form chiasmata during meiosis can result in the formation of eggs and sperm carrying an incorrect number of chromosomes, which in turn accounts for a large percentage of the miscarriages, birth defects, and infertility observed in humans [1]. Thus, DSB formation is an essential process for successful offspring production. Although it is known that DSB formation is catalyzed by Spo11, a conserved type II topoisomerase-like protein [2,3], the regulation of DSB formation is not completely understood. In this issue of *PLoS Genetics*, Lake et al. [4] shed new light on this process by identifying Trade Embargo (Trem) as a critical protein for DSB production during *Drosophila* female meiosis.

Recent studies in yeast have started to uncover the molecular basis for the regulation of DSB induction. It is known that at least ten proteins (Spo11-Ski8, Mer2-Mei4-Rec114, Rec102-Rec104, Mre11-Rad50-Xrs2) are essential for DSB induction in *Saccharomyces cerevisiae* [5]. S phase cyclin-dependent kinase Cdc28–Clb5/Clb6 (CDK-S) and the Dbf4-dependent kinase Cdc7–Dbf4 (DDK) regulate the timing of DSB formation [6,7]. Mer2 is an essential target of both CDK-S and DDK. Specifically, Mer2 is phosphorylated by CDK-S at Ser30. This phosphorylation primes Mer2 for subsequent phosphorylation by DDK on Ser29, creating a negatively charged "patch". This coordinated phosphorylation triggers the interaction of Mer2 with Mei4 and Rec114 [6,7]. CDK-S-mediated phosphorylation of Mer2 is also important for promoting the interaction between Mer2 and Xrs2 [8]. Thus, pS30 of Mer2 recruits the Mre11-Rad50-Xrs2 complex to DSB hotspots. Finally, Spo11-Ski8 and Rec102-Rec104 sub-complexes are recruited to the hotspots.

Efforts to identify DSB-inducing factors in other species have been hampered in part by the low level of sequence conservation shared with the factors first identified in *S. cerevisiae*. However, a sophisticated in silico analysis recently identified the orthologs of Mei4 and Rec114 in fission yeast, plants, and mammals [9]. Similar to yeast, *mei4−/−* mice lack meiotic DSB induction [9]. In mammals, it has been reported that Prdm9/Meisetz, which is a multi zinc-finger protein that contains KRAB and methyl transferase domains, marks DSB hotspots [10–12]. Moreover, the polymorphism of the zinc fingers alters the binding activity to hotspot sequences [10,11,12], although Prdm9 is not essential for DSB formation [13]. In other model organisms, HIM-17 which is a six THAP (C2CH) repeat containing protein in *Caenorhabditis elegans* [14], MEI1 [15] in mice, and SWI1 in *Arabidopsis thaliana* [16] have been reported as factors required for DSB formation. However, how these proteins act to make DSBs remains unclear.

Trem Is Required for DSB Formation in *Drosophila*

Trem was originally identified in a germline clone screen for meiotic mutants in *Drosophila* [17]. Trem encodes a 439 amino acid protein with an N-terminal Zinc finger Associated Domain (ZAD) and five C2H2-type zinc finger domains at the C-terminus. The original mutation alleles are $trem^{F9}$ and $trem^{f05981}$. $trem^{F9}$ carries a C to T transition, corresponding to a proline to leucine change (P299L) in the second zinc finger domain. $trem^{f05981}$ is a *pBac*-element insertion located in the gene's 5′UTR that results in a transcriptional null allele. The first analysis of $trem^{F9}$ mutants revealed a 90-fold decrease in the frequency of crossovers [17]. However, the mechanistic function for Trem in crossover formation remained unknown. In the current study, the authors suggest that Trem functions in DSB formation. They observed that Trem is expressed throughout ovariole nuclei, but is enriched in cells in region 1 of the germarium, where mitotic proliferation and premeiotic S phase take place [18]. DSBs are marked by phosphorylated histone H2AX (γH2AX) in mammals and by phosphorylated His2Av (γHis2Av) in Drosophila. Similar to *spo11/mei-W68* and *mei-P22* [19] mutants, γHis2Av foci were not observed in $trem^{F9}$ and $trem^{f05981}$ mutants. Mei-P22 forms discrete chromosome-asso-

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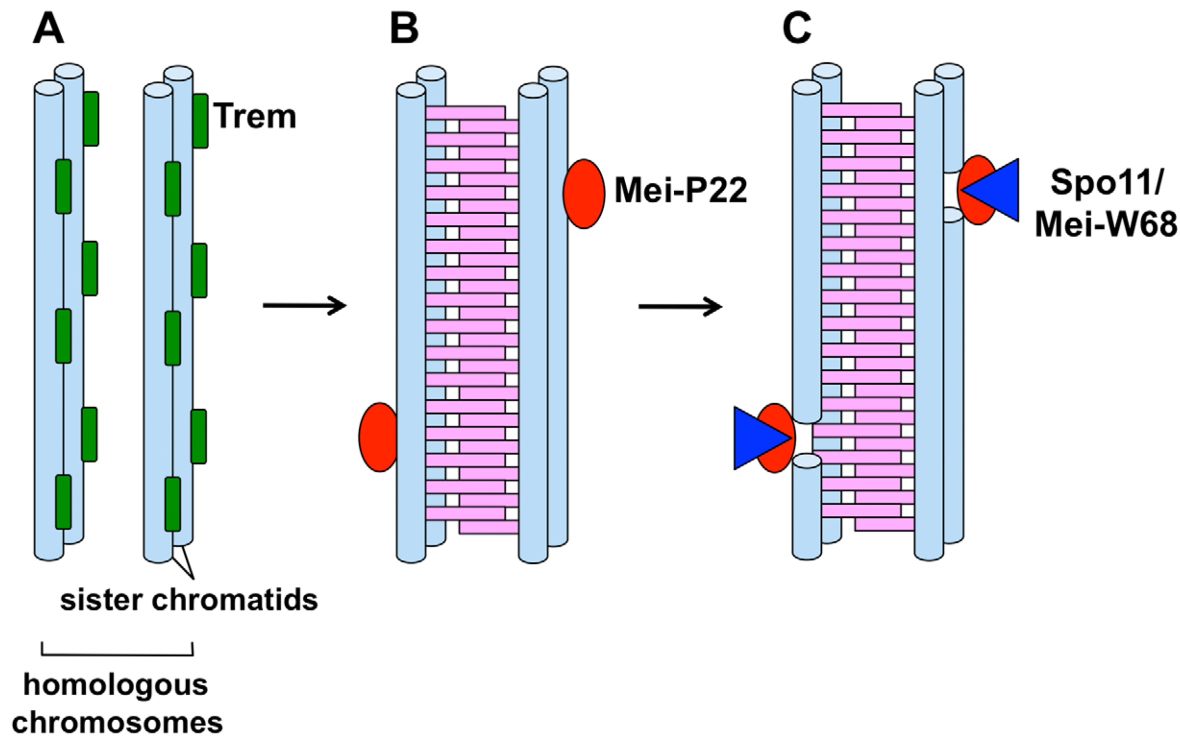

Figure 1. A model for stepwise formation of DSBs during female meiosis in *Drosophila*. (A) Thread-like localization of Trem along sister chromatids in mitotically proliferating cells (not shown) and premeiotic S phase cells (premeiotic pairing occurs at this stage). (B) Synaptonemal complex formation (pink ladder) is completed by the pachytene stage where Trem-dependent chromosome-associated Mei-P22 foci are detected. (C) DSB formation by Mei-W68/Spo11 at the Mei-P22-marked sites.
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ciated foci during early prophase that are thought to mark the position of future DSBs [19,20]. Intriguingly, Mei-P22 foci disappeared in *trem* mutants, suggesting that Trem is required to localize Mei-P22 to discrete foci during early prophase. Two strong lines of evidence are presented which support the idea that DSBs are not induced in *trem* mutants: 1) *trem* mutations suppress the defects in egg shell formation, karyosome formation, and oocyte selection detected in *spn-B/rad57* and *okr/rad54* DNA repair mutants; 2) X-ray exposure, which introduces exogenous DSBs, partially suppresses the defects in γHis2Av formation and meiotic spindle formation observed in *trem* mutants. These results suggest that Trem is required for DSB formation during meiotic prophase (Figure 1).

Setting the Stage for DSB Formation

How does Trem contribute to DSB formation? In *Drosophila*, homologous chromosome pairing occurs during the mitotic proliferation stage. This phenomenon, combined with the expression pattern of Trem, leads to the hypothesis that the preparation for DSB formation also occurs before meiotic prophase. One possibility is that Trem somehow changes the chromatin state via its DNA binding activity and an unknown interacting partner. After premeiotic replication, Mei-P22 is recruited to the DSB sites. Intriguingly, Mei-P22 has nine potential CDK target sites. Investigation into Mei-P22 regulation by CDK might therefore be very revealing. Although Trem does not harbor a methyl transferase domain and its localization pattern looks evenly distributed on the chromosomes, it will be important to assess whether Trem acts similarly to Prdm9 because Trem is also a multi zinc-finger protein. Moreover, specific posttranslational modifications of Trem may localize Trem to the DSB sites that are the future locations occupied by Mei-P22. Indeed, analysis through the Netphos, SUMOsp, and BDM-PUB programs identify several potential phosphorylation, ubiquitination, and sumoylation sites on Trem. Future studies will therefore reveal how Trem conditions or primes the chromatin state for recruitment of Mei-P22 to the DSB sites.

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