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Signaling-mediated meiotic recombination in plants

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Meiotic recombination provides genetic diversity in populations and ensures accurate homologous chromosome segregation for genome integrity. During meiosis, recombination processes, from DNA double strand breaks (DSBs) to crossover formation are tightly linked to higher order chromosome structure, including chromatid cohesion, axial element formation, homolog pairing and synapsis. The extensive studies on plant meiosis have revealed the important conserved roles for meiotic proteins in homologous recombination. Recent works have focused on elucidating the mechanistic basis of how meiotic proteins regulate recombination events via protein complex formation and modifications such as phosphorylation, ubiquitination, and SUMOylation. Here, we highlight recent advances on the signaling and modifications of meiotic proteins that mediate the formation of DSBs and crossovers in plants.

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Introduction

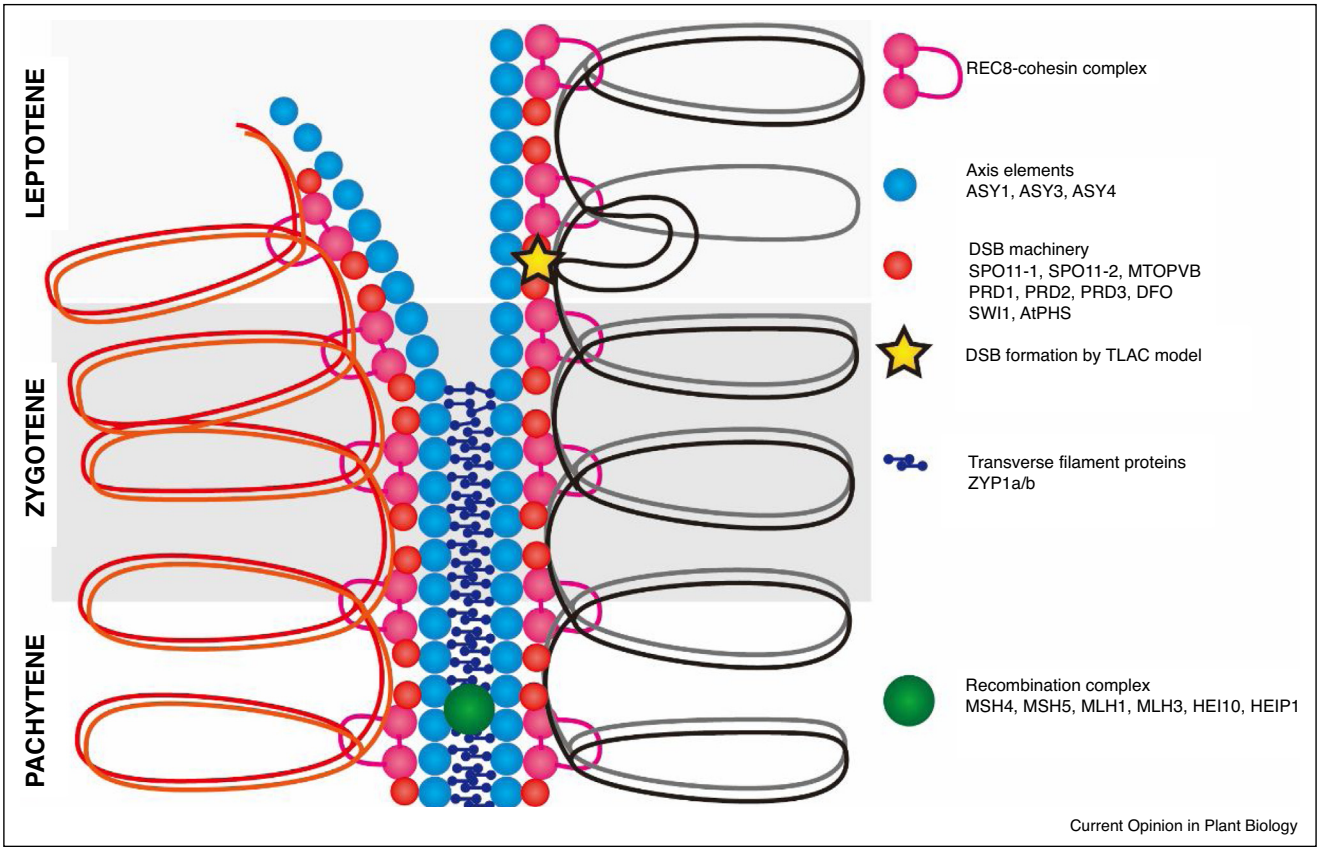
Meiosis creates new allelic combinations and maintains genome ploidy over generations in sexually reproducing eukaryotes [1–3]. Meiotic cells undergo two consecutive chromosome segregations (meiosis I, II) following a single DNA replication. During meiosis I, meiotic recombination initiates with the induction of programmed DNA double strand breaks (DSBs) by an evolutionally conserved topoisomerase VIA-like SPO11 [4]. At DSB sites, 5′ ends of DNA are processed and resected to produce 3′ tails of single-strand DNAs (ssDNAs). Bacterial RecA homolog proteins, DMC1 and RAC51, lead ssDNAs to invade either sister or non-sister chromatids for homology search. The non-sister chromatid invading molecules can be resolved as non-crossovers or crossovers via different DNA repair strategies. Two DNA repair pathways result in reciprocal crossovers [1,5,6]. One is an interfering pathway (class I), leading to approximately 80–85% crossovers. The other is a non-interfering pathway (class II), representing 10–15% of crossovers. In *Arabidopsis*, the class I crossovers are generated by ZMM proteins (ZIP4, MSH4, MSH5, MER3, HEI10, SHOC1, PTD, MLH1, MLH3) and sensitive to crossover interference in which one crossover interferes with formation of another crossover. Class II crossovers are non-interfering and limited by anti-crossover factors [1,5,6].

Meiotic recombination processes are spatiotemporally associated with higher-order chromosome structure [3]. Following DNA replication, pairs of sister chromatids form the meiotic chromosome structure with a series of chromatin loops that are anchored at their bases to the chromosome axes (Figure 1). Meiotic DSBs occur in the developing chromosome structural loops tethered at axial elements [7]. DSBs promote pairing of homologous chromosomes (homologs), and lead to a tripartite structure called the synaptonemal complex (SC) at pachytene. The SC assembly is required to stabilize paring interactions of homologs and promotes interhomolog crossover formation. In plants and other organisms, the conserved meiotic proteins involved in recombination events and chromosome organization have been identified [1,8]. Recent works have revealed the roles of meiotic proteins in linking recombination steps to chromosome structural changes via protein complex formation and modifications such as ubiquitination [8]. This review summarizes the studies on regulation of meiotic recombination by complex formation and post-translational modifications of proteins in plants, particularly with focus on *Arabidopsis*.

A conserved catalytic subcomplex in meiotic DSB machinery

The evolutionally conserved SPO11 transesterase catalyzes the formation of meiotic DSBs [4,7]. Two SPO11 monomers are required to induce a single DSB since the catalytic tyrosine residue of each SPO11 is covalently attached to 5′ ends of DNA at a DSB site. Then, endonucleolytic processing close to the DSB site occurs, producing ssDNAs and SPO11-oligonucleotide complexes as DSB byproducts. The ssDNAs undergo downstream repair processes. The SPO11-oligonucleotides can be purified and sequenced to make high-resolution DSB maps [9••,10,11]. A single gene encodes the SPO11 monomer in the yeasts, *Saccharomyces cerevisiae* (*SPO11*) and *Schizosaccharomyces pombe* (*REC12*). However, plants have three SPO11 paralogs, SPO11-1, SPO11-2 and SPO11-3. In *Arabidopsis*, the SPO11-1 and SPO11-2 are non-redundantly required for

Figure 1



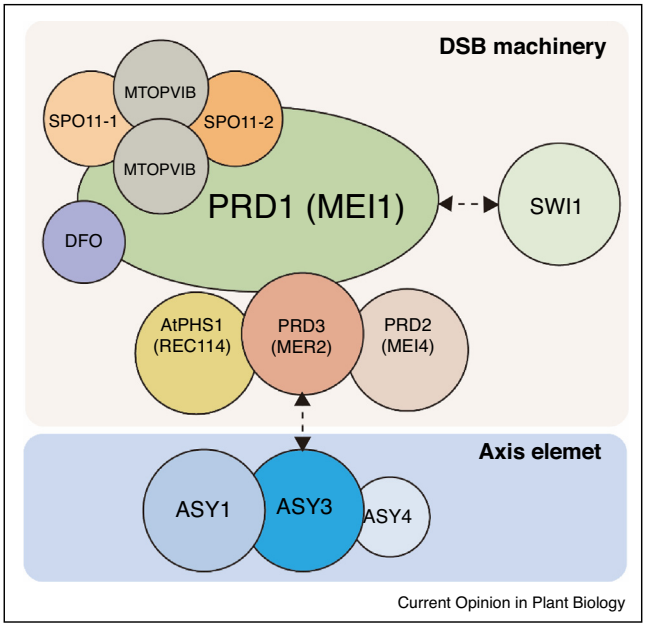
Assembly of meiotic protein complexes and meiotic chromosome in plants.
At leptotene, chromosomes comprise a series of chromatic loops and the proteinaceous axial elements enriched by REC8-cohesin complex and complexes of coiled-coil proteins ASY1, ASY3, and ASY4. During the axis element occurrence, PRD3 and its phosphorylation may mediate the loading of other DSB proteins on the chromosome axes. The DSBs form on loop chromatin by the TLAC model. The meiotic DSB formation induces DSB signaling and the phosphorylation of ASY3 and ASY1, which leads them to interact with ZYP1a/b for completing SC formation from zygotene to pachytene stage. HEI10 signaling mediates crossover designation and formation via MSH5, HEIP1 and potentially other meiotic proteins. According to the studies on other organisms, potential phosphorylated proteins are REC8 [38,40], ASY1 [31••], ASY3 [31••], PRD3/MER1 [35], ZYP1a/b [63], and MSH4 [64]. Potential proteins for ubiquitination are HEIP1, MSH5 and ZIP4 [54•]. Potential protein for SUMOylation is ASY3 [41,42]. Abbreviations: PRD1/2/3, putative recombination initiation defect 1/2/3; SPO11, sporulation11; ASY1/3/4, ASYNAPTIC 1/3/4; MTOPVIB, meiotic topoisomerase VIB-like; DFO, DSB forming; SWI1, SWITICH1; AtPHS1, Arabidopsis thaliana POOR HOMOLOGOUS SYNAPSIS1; TLAC, tethered loop axis complex; MSH4/5, MutS Homolog 4/5; MLH1/3, MutL Homolog 1/3; HEI10, Human Enhancer of cell Invasion N°10; HEIP1, HEI10 Interaction Protein 1.

meiotic DSB formation while SPO11-3 is involved in endoreduplication in somatic cell division [12–14]. The SPO11-1 and SPO11-2 are structurally similar to subunit A of archaeal DNA topoisomerase VI complex and form a hetero-tetrameric complex (A2B2) with meiotic topoisomerase VIB-like protein (MTOPVIB) [15••]. The tetrameric complex formation of SPO11-1, SPO11-2 and MTOPVIB is essential for DSB formation in *Arabidopsis* and the assembly of catalytic complex appears to be structurally conserved in eukaryotes (Figure 2) [16••]. Sequencing of the short oligonucleotides (~30–50 nt) attached to SPO11-1 revealed that *Arabidopsis* DSB hotspots occur at nucleosome-depleted gene regulatory regions and specific DNA transposons [9••,17••]. On the basis of the tetrameric complex model for SPO11 activity, SPO11-2-oligonucleotide maps will be predicted to show a similar pattern to SPO11-1. However, it is possible that SPO11-2 may show a specific DSB pattern in plants since it was shown that only SPO11-1 but not SPO11-2 is essential for MTOPVIB loading to chromosomes [15••].

The assembly of meiotic DSB machinery and signaling

Like other sexual reproducing organisms, *Arabidopsis* SPO11 activity requires accessory proteins for meiotic DSB formation [1]. PRD1, PRD2, and PRD3 are the orthologs of MEI1, MEI4, and MER2/IHO1 respectively, while DFO, a coiled-coil protein is plan-specific. PRD1, the ortholog of human MEI1 acts as a scaffold protein that interacts with PRD2/MEI4, PRD3/MER2, DFO and

Figure 2



Meiotic DSB complex and axial element components in plants. In *Arabidopsis*, meiotic DSB machinery consists of SPO11-1, SPO11-2, MTOPVIB, PRD1, PRD2, PRD3, and DFO. SPO11-1, SPO11-2 and MTOPVIB form a tetrameric catalytic subcomplex, like the DNA topoisomerase VI complex [16••]. PRD1, the ortholog of mouse MEI1 mediates the interactions of the catalytic subcomplex with PRD3 and DFO [19•]. It is unclear yet whether SWI1 and AtPHS1 can interact directly with DSB machinery components. In axial element, ASY3 interacts with ASY1 and ASY4. ASY1 was coimmunoprecipitated with PRD3 [31••,32], indicating the association between DSB machinery and axis element. Abbreviations: MEI1, Meiotic Double-Stranded Break Formation Protein 1; MEI4, Meiotic Double-Stranded Break Formation Protein 1; MER2, meiotic recombination 2 protein; REC114, Recombination 114; SPO11, sporulation11; MTOPVIB, meiotic topoisomerase VIB-like; DFO, DSB forming; SWI1, SWITICH1; AtPHS1, Arabidopsis thaliana POOR HOMOLOGOUS SYNAPSIS1; ASY1/3/4, ASYNAPTIC 1/3/4.

catalytic complex components, SPO11-1, SPO11-2 and MTOPVIB (Figure 2) [18,19•]. Hence, the assembly of the DSB machinery into protein complexes is required for DSB formation in *Arabidopsis*. It is unclear whether other DSB proteins, SWI1/DYAD and AtPHS1 (REC114 homolog) are involved in the DSB machinery assembly in *Arabidopsis* [20,21]. Protein interaction assays or SPO11-1-oligonucliotide detection in DSB mutants will help to clarify their roles in meiotic DSB formation as shown in *prd2* [9••,10,19•].

Notably, *Arabidopsis* PRD3 was shown to be the ortholog of MER2 that is evolutionally conserved among species [22••]. MER2 and its phosphorylation by cyclin-dependent kinases facilitates meiotic recombination initiation by mediating the subcomplex formation with MEI4/PRD2 and REC114/AtPHS1 on chromosome axes in yeast and fungi [7,22••,23–25]. MER2 is recruited to axes and its phosphorylation promotes the recruitment of its interactors, MEI4 and REC114, which may direct SPO11 and other DSB proteins to chromosome axes. Thus, before DSB formation it appears that the DSB machinery containing SPO11 and its accessory proteins is loaded and assembled on the chromosome axes enriched by the REC8-cohesin complex and axis proteins in plants (Figure 1), like yeast. It is likely that MER2/PRD3 mediates the assembly of pre-DSB recombination complexes including SPO11 to axes for DSB formation in *S. cerevisiae* and *Sordaria* (Figure 2) [7,22••,24]. However, the roles for phosphorylation and axial location of PRD3 in DSB formation remain unexplored yet in *Arabidopsis*. To elucidate the relationship of meiotic DSB proteins and chromosome axis during meiotic DSB formation in plants, extensive studies and ChIP-seq experiments on meiotic DSB proteins and axis proteins in *Arabidopsis* will be necessary [26••].

In *S. cerevisiae*, tethering chromatin loops (sites of DSB formation) to axes (locations of DSB proteins) is mediated by protein interactions between Mer2 (DSB protein at axis)-Spp1 (shared interacting protein)-H3K4 trimethylation (histone modification at chromatin loop), as the proposed tethered loop-axis complex (TLAC) model [7,24]. H3K4 trimethylation (H3K4m3) promotes DSB formation and affects DSB distribution in budding yeast and mammals; however, how H3K4m3 influences DSB and crossover formation remains an intriguing open question in plants. The signaling of ATM kinase inhibits meiotic DSB formation in mouse and *S. cerevisiae* [27,28]. *Arabidopsis atm* mutants are partially sterile, with normal crossover frequency, but the role of *Arabidopsis* ATM in meiotic DSB formation and crossovers is still unclear and needs to be explored in further detail [29].

SC proteins and posttranslational modifications

Like *S. cerevisiae*, *Arabidopsis* axial elements are assembled along chromosomes and then axes from the homologous chromosomes are connected by the polymerization of the central element of the SC, forming the lateral elements (Figure 1) [6,30]. ASYNAPTIC1 (ASY1), ASY3, ASY4, and the cohesin complex including REC8/SYN1 were identified as the axial elements in *Arabidopsis* [6,31••,32,33]. ASY1 and ASY3, coiled-coil proteins interact with each other and are likely to be the functional homologs of yeast Hop1 and Red1 respectively [33,34]. ASY4 is a small protein and shares a similar coiled-coil domain with ASY3 [32]. ASY4 interacts with ASY3 *in vivo* but not with ASY1, indicating that ASY3 mediates the interactions among ASY1, ASY3 and ASY4 (Figure 2). ASY4 was also included in the list of *Brassica oleracea* ASY1-associated proteins identified by affinity proteomics [31••]. Intriguingly, key meiotic proteins, PRD3/MER2, DMC1, CDK1 homolog, CDKA;1, TOP2, ICU2 (the catalytic subunit of DNA polymerase

α), PCH2, ZYP1a as well as the components of the cohesion complex and proteasome components (see below) were coimmunoprecipitated with ASY1 [31••]. Notably PRD3/MER2, an essential DSB protein was at first shown to be associated with the axial elements in plants by the affinity proteomics. However, it remains unexplored whether PRD3 binds directly to ASY1. Together, the proteomic data suggest the importance of protein interconnections during meiotic recombination progress in plants.

Affinity proteomics and mass spectrometry also identified multiple phospho-modified residues of *Bo*ASY3 and *Bo*ASY1 as shown in Red1 and Hop1 of the budding yeast [31••,35,36]. The phosphorylation of chromosome axis proteins including Red1, Hop1 and Rec8 is important for their activity during meiosis [36–39]. Hop1 phosphorylation is mediated by ATM/ATR kinases, which subsequently activates Mek1 kinase for crossover formation in yeast [35]. The phosphorylation of *Bo*ASY3 and *Bo*ASY1 suggests that the phosphorylation signaling may play a crucial role in regulating meiotic recombination in plants. In yeast, Rec8 phosphorylation is required for its cleavage along chromosome arms during meiosis but Rec8 continues to localize at the centromeres up to metaphase II. In yeast, the centromeric Rec8 is dephosphorylated by protein phosphatase 2A (PP2A) and protected from cleavage [38,39]. It was recently shown that *Arabidopsis* PP2A B′α and β are required to maintain the centromeric sister chromatin cohesion [40].

Red1, a functional homolog of *Arabidopsis* ASY3 is also modified by SUMO (small ubiquitin-like modifier) in budding yeast [41,42]. The SUMOylation of Red1 depends on Zip3, a SUMO E3 ligase and occurs during early meiosis, coincidently with the appearance of SC. Red1 SUMOylation drives Red1 to interact with Zip1, the component of transverse filaments of SC and thereby promotes to zip up SC. Consistently, SUMOylation of Red1 is involved in the crossover interference pathway [43]. In plants it remains unexplored whether ASY3 can be modified by SUMOylation as a functional analog of Red1. In *Arabidopsis*, SUMO proteins locate on chromosome axes during meiosis and the mutation of *MMS21* encoding a SUMO E3 ligase causes chromosome fragmentation and mis-segregation, suggesting the meiotic roles for SUMOylation [44,45]. It has been shown in other organisms that the SUMOylation-ubiquitin-proteasome system plays an important role in regulating meiotic recombination [8,46••,47]. The system is likely conserved in plants, nonetheless it will be interesting to explore how it can control meiotic recombination by modulating plant meiotic SC proteins.

Ubiquitin and ubiquitin-like protein signaling-mediated meiotic recombination

In *Arabidopsis*, HEI10, a RING finger containing protein which may have an ubiquitin E3 ligase activity or SUMO E3 ligase activity or both, is required for class I crossover formation and controls crossover number in a dosage-dependent manner [48,49••,50]. Increasing *HEI10* transcript level by introducing extra copies of *HEI10* transgene was sufficient for elevating crossovers, with a correlation between transcript level and crossover number [49••]. *Arabidopsis* HEI10 is similar to the mammalian protein HEI10 that shows an E3 ubiquitin ligase activity [46••], although it is unclear whether *Arabidopsis* HEI10 has the E3 ubiquitin ligase activity *in vivo*. In *Arabidopsis* and rice, HEI10 foci mark the sequential chromosome structural changes from ASY1, ZPY1 to MLH1 signals during meiosis [48,51]. In *Arabidopsis*, higher temperatures can increase HEI10 foci number per meiotic cell and crossover frequency in defined intervals via class I pathway [52•,53•]. The data suggest that temperature may affect *HEI10* transcript level or HEI10 protein stability. HEI10 Interaction Protein 1 (HEIP1) was identified as a HEI10 interacting partner in rice [54•]. The rice HEIP1 interacts with ZIP4 and MSH5 that promote class I crossover formation. Thus, HEI10 may mediate ubiquitin or SUMO-signaling directly via HEIP1, ZIP4, and MSH5 in plants. Interestingly, an F-box ubiquitin E3 ligase is required for DSB formation and meiotic progression in rice [55•]. This suggests that the ubiquitin-proteasome system may play critical roles in meiotic recombination from DSBs to crossover formation in plants. In *Arabidopsis*, crossover formation appears to be rather sensitive to DSB activity and temperature changes despite of the crossover homeostasis [56•,57]. It is likely that the ubiquitination system may enable plants to sensitively control meiotic recombination in response to the changes of internal and external stimuli. It is also notable that the neddylation protein modification pathway regulates meiotic crossover distribution in *Arabidopsis*, probably via ubiquitination and proteasome systems [58,59]. In the *axr1* mutant where a key component of neddylation E1 enzyme complex is disrupted, the average number of total crossovers is unchanged but interestingly class I crossovers tend to cluster together along chromosomes at pachytene [58]. The crossover clustering and mislocalization in *axr1* appear to be involved in defects in synapsis, changing interference. Since neddylation is known to activate subclasses of Cullin-RING ubiquitin ligases, it would be interesting to elucidate how neddylation, ubiquitination and proteasome systems control meiotic crossover pattern via modifying meiotic proteins and their interplays in plants.

Conclusions

Meiotic protein complex formation and modifications have been found to link changes in chromosome dynamics with meiotic recombination. In the last decade, the genetic and molecular studies on meiotic recombination have provided great strategies for plant breeding [1,5,60]. For example, the strategy for disrupting anti-crossover factors has been translated to crops [61], and the

replacement of meiosis by mitosis contributed to the generation of rice clonal F1 hybrid progeny [62]. However, there are still many interesting questions to be answered about molecular mechanisms of meiosis in plants. Is it possible to further increase crossovers in plants by discovering new crossover modulators beyond the known anti-crossover factors and HEI10? Can plant class I or class II crossovers be increased or decreased effectively by modulating meiotic DSB formation or SC dynamics? The understanding on signaling and posttranslational modifications of plant meiotic proteins may help to answer these questions. Conventional proteomics is useful to elucidate meiotic proteins and their modifications [31••]. Other approaches such as protoplast expression systems could be utilized to test whether meiotic proteins are modified by phosphorylation, SUMOylation or ubiquitination. High-throughput measurements of crossover rate by using fluorescent seeds or pollens can allow to perform genetic screening to find new factors controlling crossover frequency and distribution in plants [49••]. Of course, it is also worth noting that the protein modification and signaling studies on plant meiosis provide insights into elucidating fundamental mechanism of meiosis across species.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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