Understanding and Manipulating Meiotic Recombination in Plants[OPEN]

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Meiosis is a specialized cell division, essential in most reproducing organisms to halve the number of chromosomes, thereby enabling the restoration of ploidy levels during fertilization. A key step of meiosis is homologous recombination, which promotes homologous pairing and generates crossovers (COs) to connect homologous chromosomes until their separation at anaphase I. These CO sites, seen cytologically as chiasmata, represent a reciprocal exchange of genetic information between two homologous nonsister chromatids. This gene reshuffling during meiosis has a significant influence on evolution and also plays an essential role in plant breeding, because a successful breeding program depends on the ability to bring the desired combinations of alleles on chromosomes. However, the number and distribution of COs during meiosis is highly constrained. There is at least one CO per chromosome pair to ensure accurate segregation of homologs, but in most organisms, the CO number rarely exceeds three regardless of chromosome size. Moreover, their positions are not random on chromosomes but exhibit regional preference. Thus, genes in recombination-poor regions tend to be inherited together, hindering the generation of novel allelic combinations that could be exploited by breeding programs. Recently, much progress has been made in understanding meiotic recombination. In particular, many genes involved in the process in Arabidopsis (*Arabidopsis thaliana*) have been identified and analyzed. With the coming challenges of food security and climate change, and our enhanced knowledge of how COs are formed, the interest and needs in manipulating CO formation are greater than ever before. In this review, we focus on advances in understanding meiotic recombination and then summarize the attempts to manipulate CO formation. Last, we pay special attention to the meiotic recombination in polyploidy, which is a common genomic feature for many crop plants.

MEIOTIC RECOMBINATION: FROM DNA DOUBLE-STRAND BREAKS TO CROSSOVER FORMATION

Meiosis consists of two rounds of chromosome segregation, in which homologous chromosomes separate during meiosis I and sister chromatids further separate during meiosis II (Fig. 1). The accurate segregation of homologous chromosomes requires meiotic recombination that is initiated by the formation of DNA double-strand-breaks (DSBs) and is completed by repair of these breaks. One of the outcomes is crossover (CO) formation, during which a subset of DSBs are repaired using homologous chromosome as a template. The remaining DSBs are repaired without reciprocal exchange to form noncrossovers (NCOs) or are repaired by sister chromatids. Several comprehensive review articles devoted to the molecular mechanisms of meiotic recombination have been published in recent years (Lam and Keeney, 2014; Luo et al., 2014; Mercier et al., 2015). In this section, we give a brief overview of meiotic recombination with a focus on recent findings (Fig. 1; Box 1). In contrast to increasing CO number, strategies to eliminate COs are discussed in Box 2.

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Formation of DNA DSBs

The programmed formation of DSBs is catalyzed by a SPO11-containing complex that is thought to be structurally similar to the archaeal topoisomerase VI complex (Bergerat et al., 1997). In Arabidopsis (*Arabidopsis thaliana*), it consists of two A subunits (SPO11-1 and SPO11-2) and the recently identified B subunit (MTOPVIB), likely forming a heteromeric complex (Stacey et al., 2006; Vrielynck et al., 2016). In budding yeast (*Saccharomyces cerevisiae*), around 10 SPO11 accessory proteins have been identified for DSB formation (Lam and Keeney, 2014). In plants, besides the SPO11 core complex, several proteins (PRD1, PRD2, PRD3, DFO, CRC1, and COMET) essential for break formation have been identified. These probably fulfill analogous roles to some of the SPO11 accessory proteins in other species; for example, PRD1 has some similarities to Mei1 in mouse (De Muyt et al., 2007) and PRD2 is a possible ortholog of Mei4 from mouse and *S. cerevisiae* (De Muyt et al., 2009; Kumar et al., 2010). In contrast, PRD3 and DFO appear to be plant specific (Zhang et al., 2012). In addition, rice (*Oryza sativa*) CRC1, which shares similarities with PCH2 in Arabidopsis and *S. cerevisiae* was shown to be required for DSB formation (Miao et al., 2013), but there is no detectable change in DSB number in *pch2* mutants in Arabidopsis (Lambing et al., 2015). Interestingly, P31[comet] was identified recently as a protein interacting with rice

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- Identification of the MTOPVIB suggests that SPO11 catalyzes DSBs in a complex that structurally resembles the ancestral topoisomerase complex.

- CO hotspots in Arabidopsis are associated with active chromatin features such as H2A.Z, H3K4me3, low DNA methylation, and low nucleosome density.

- CO formation is actively repressed by distinct protein complexes. These anti-recombination proteins can be targeted by mutagenesis approaches to increase the number of COs.

- The distribution of COs is altered upon temperature increase, leading to a greater number of interstitial/distal COs.

- Studies in autotetraploids are providing intriguing insights into the relationship between chromosome axis proteins and CO control.

- Allotriploid Brassica exhibits a remarkable elevation in the level of recombination.

BOX 1: Meiotic Mutants Affecting CO Positioning

- Several genes that influence the frequency and distribution of COs have been identified in Arabidopsis. They can be grouped into three classes based on their functions: (1) epigenetic mark deposition, (2) anti-CO enzymes, and (3) other regulators.

- (1) Mutants such as *met1* and *ddm1* defective in DNA methylation maintenance cause extensive hypomethylation in the genome. These epigenetic changes result in the redistribution of COs, with pericentromeric decreases and distal euchromatic increases in recombination. Surprisingly, reduced methylation levels in pericentromeric regions that supposedly increase chromatin accessibility did not result in more proximal COs. The total CO number remains unchanged, suggesting that DNA methylation influences CO positioning but not CO number (Melamed-Bessudo and Levy, 2012; Mirouze et al., 2012; Yelina et al., 2012, 2015).

- (2) DNA helicase and topoisomerases, such as FANCM, TOP3a, and RECQ4A/B, limit CO formation, probably by unwinding and resolving joint molecules to promote NCOs. In these mutants, extra class II COs that depend on MUS81 are distributed in the distal regions (Crismani et al., 2012; Knoll et al., 2012; Séguéla-Arnaud et al., 2015), whereas class I COs seem to be unaffected.

- (3) Three other proteins have been reported to mediate CO positioning: (1) PCH2, an AAA+ ATPase, is thought to remodel chromosomal axes and is responsible for ASY1 removal (Lambing et al., 2015); (2) AXR1, an E1 enzyme of the neddylation complex, functions in a posttranslational protein modification pathway with unclear targets (Jahns et al., 2014); and (3) PSS1, a kinesin-like protein, is likely to be a component of the chromosome mobility machinery (Duroc et al., 2014). Interestingly, mutants of these genes show an altered pattern of COs combined with defective synapsis. DSBs appear to form normally, but CO interference is altered in all three mutants in some chromosomal regions. Although the mechanisms underlying CO interference affected by these genes remain elusive, the common defect in synapsis in the three mutants suggests a potential role of complete synapsis in regulating the maturation of recombination intermediates into COs. Loss of this regulation results in the mislocalization of class I COs. Except for decreased COs in the *pch2* mutant, the number of COs remains unaffected in *axr1* and *pss1* mutants.

CRC1 (Ji et al., 2016). Similar to CRC1, P31^{comet} also is essential for DSB formation in rice. These differences suggest diverse regulation of DSB formation among species.

DSBs are potentially hazardous to cells; on the other hand, meiotic cells require DSBs to generate recombination. Thus, meiotic DSB formation must be tightly controlled at the right time and in the proper places. Studies in *S. cerevisiae* and mouse have shown that DSBs do not occur randomly in the genome; instead, they form preferentially in regions of a few kilobases, called DSB hotspots (Baudat and Nicolas, 1997; Smagulova et al., 2011). At the chromatin scale, DSB hotspots in *S. cerevisiae* are located at gene promoters, which are strongly associated with low-nucleosome-density regions and are enriched with the histone H3 Lys-4 trimethylation (H3K4me3), an open chromatin mark (Pan et al., 2011). In contrast, DSBs in mammals are located in intergenic regions but also marked with H3K4me3 (Smagulova et al., 2011). These observations indicate that chromatin accessibility is an important feature for DSB formation. In plants, DSB positioning at the

chromatin or DNA sequence scale has not yet been explored due to the difficulty of obtaining meiotic cells in sufficient quantity to perform the studies. One additional component that plays an important role in DSB formation is the meiotic chromosome axial element,

BOX 2: Strategies to Eliminate COs

Apart from increasing COs in recombination-poor regions, elimination of COs also has many breeding applications. For example, suppression of meiotic COs in an elite hybrid can allow the transmission of nonrecombinant chromosomes to the next generation. Two methods have been developed for this purpose using meiotic mutants in combination with the centromere-mediated genome elimination method (Ravi and Chan, 2010). The first approach, named reverse breeding, uses an RNA interference:*DMC1* construct to suppress CO formation in a hybrid. DMC1 is required for the interhomolog strand invasion during recombination, and loss of DMC1 causes complete univalents in Arabidopsis. The resulting nonrecombinant haploid gametes with balanced chromosome set are fertilized with pollen from the *cenh3* GFP tailswap line, in which the N-terminal tail of CENH3 was replaced by the tail of the H3.3 variant fused with GFP (Wijnker et al., 2014). Chromosomes from males are eliminated to generate nonrecombinant haploid plants. Double haploid plants are selected from the rare seeds following self-pollination of the haploids.

Finally, crossing two complementary lines allows production of the hybrid (Wijnker et al., 2012). The second method, called MiMe, turns meiosis into mitosis to produce nonrecombinant diploid gametes. In this synthetic approach, triple mutants of *rec8/syn1*, *spo11-1*, and *osd1* are used so that meiotic recombination and second meiotic division are omitted. The mutation in the *rec8/syn1* gene encoding a meiotic cohesion protein abolishes the formation of meiotic chromosome axes, and additionally, DSBs fail to be repaired. In combination with the *spo11-1* mutant, the DSB-dependent meiotic recombination process is abolished. Addition of the *osd1* mutation that skips the second meiotic division transforms meiosis into mitosis-like division, resulting in nonrecombinant diploid gametes (d'Erfurth et al., 2009). Finally, Arabidopsis clonal seeds can be obtained by crossing the triple mutant with the *cenh3* GFP tailswap line (Marimuthu et al., 2011). However, it remains to be examined whether these methods can be transferred into crops.

which assembles before DSB formation to organize chromatins into linear arrays of loops (Fig. 1). Studies in *S. cerevisiae* have suggested that DSB sites located within chromatin loops are tethered onto chromosome axes where most DSB accessory proteins reside (Panizza et al., 2011). This tethering is mediated by Spp1p, which interacts with H3K4me3 and the axial protein Mer2 (Sommermeyer et al., 2013). The nucleosome-depleted regions near the tethered segment (marked with H3K4me3) become accessible to SPO11. In mouse, the zinc finger protein PRDM9 binds with DSB sites and catalyzes H3K4me3, which are predicted to be tethered to the axis for break formation (Lam and Keeney, 2014). This chromatin-tethering process has not been investigated in plants. However, some plant axial proteins are required for full DSB levels, suggesting that the axial element may be involved in DSB formation in plants.

DSB Repair and Their Fates

Following DSB formation, DNA ends are nicked and resected by the MRE11-RAD50-NBS1/XRS2 complex to generate 3′ ssDNAs (Neale et al., 2005). These ssDNA nucleofilaments associated with DMC1 and RAD51 recombinases search for homologous sequences (Bishop et al., 1992; Sung and Robberson, 1995). The ssDNA may invade either the sister chromatid or homologous chromatids. This latter event is thought to serve as the major process contributing to the juxtaposition of homologous chromosomes (i.e. homologous pairing or alignment). Repair of these interhomolog invasion events produces either COs or NCOs. How and when the decision between CO versus NCO is made remains unclear, but studies have shown that it is controlled at many levels. First, the formation of at least one CO per homolog pair is required for the accurate segregation of homologous chromosomes, termed the obligate CO (Jones and Franklin, 2006). Second, the presence of a CO decreases the probability of a second CO occurring nearby, termed CO interference (Sturtevant, 1915). Finally, CO homeostasis, which has been documented in *S. cerevisiae* and mouse, can maintain a stable number of COs even when DSB numbers are altered (Martini et al., 2006). However, a recent study suggested that, at least in maize (*Zea mays*), CO homeostasis is not prominent (Sidhu et al., 2015), and this result may argue that plants in general do not exhibit this phenomenon.

CO Formation

Following strand invasion, the 3′ end-invading strand serves as a primer for DNA synthesis, leading to unwinding of the double-stranded homologous DNA to form a displacement loop structure (D-loop; Petukhova et al., 1998). This early intermediate can give rise to many forms of joint molecules, and differential stabilization and resolution of joint molecule intermediates seem to determine the fate of recombination events (Allers and Lichten, 2001). If the D-loops are extended, which may better expose the displaced strand, the second end of DSBs can anneal with the displaced strand of the D-loop in a process called
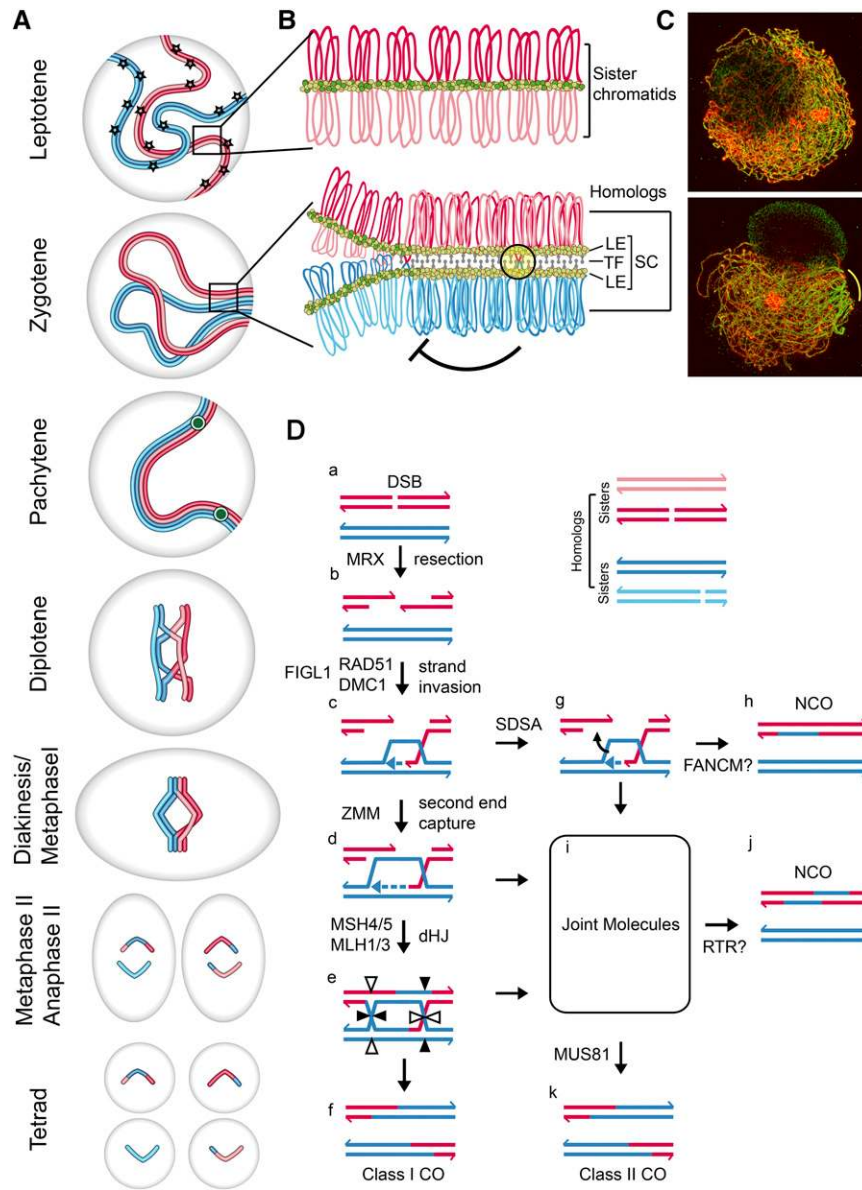
Figure 1. Meiotic recombination. A, A meiotic cell with one pair of homologous chromosomes is illustrated (marked as red and blue); each consists of two newly replicated sister chromatids (e.g. dark and light red). Meiotic recombination starts with the formation of double-strand breaks (DSBs; asterisks) at leptotene. DSB sites serve as probes for homology searches to facilitate homologous pairing. At zygotene, synapsis is initiated between prealigned regions of homologous chromosomes that often occur in subtelomeric regions in plants with large genomes. Crossovers (COs; circles) are matured with a strong regional bias at pachytene and are seen as chiasmata at diplotene. The formation of COs ensures that homologous chromosomes stay together until their separation at metaphase I. Sister chromatids segregate further at metaphase II. B, Meiotic chromatids are organized in loops with their bases anchored on chromosome axes (green circles). DSBs are formed in the context of the chromatin loop-axis conformation. The chromatin structure and axial proteins influence DSB formation presumably by regulating the accessibility and activity of the DSB machinery. It is still poorly understood how DSBs are placed, catalyzed, and processed in relation to the loop-axis conformation. As homologous chromosomes synapse with the installation of transverse filaments (TF) between homologous axes (lateral elements [LE]) at zygotene, recombination intermediates, seen as recombination nodules, are located in the central region of the synaptonemal complex (SC). Once a CO is designated (marked as a light yellow circle), unclear mechanisms promote the repair of the remaining recombination intermediates toward noncrossovers (NCOs). The axial protein is remodeled upon SC formation, which may be involved in the process. C, Maize nuclei at late leptotene (top) and zygotene (bottom) stages are shown with chromatin (red) and DSY2 (green) staining by superresolution microscopy. Note that SC formation starts mostly in distal chromosomal regions where telomeres cluster (indicated as an arc). D, DSBs can be generated in all four chromatids (right), and only one chromatid from each homolog is shown (a; red and blue double lines represent double-stranded DNA). DSBs are processed to yield 3′ end single-stranded DNA (ssDNA; b) and invade the homologous chromatids with the assistances of RAD51

second end capture annealing. After gap filling and ligation, a unique heteroduplex DNA configuration called the double Holliday junction (dHJ) can be formed (Schwacha and Kleckner, 1995). This dHJ can be resolved to form either an NCO or a CO. These dHJ-dependent COs, termed class I COs, are subjected to interference, so they are farther apart along the chromosome than expected by random distribution. Genetic and molecular evidence has shown that the formation of class I COs depends on a group of proteins initially identified in *S. cerevisiae* and collectively named ZMM proteins. They include Zip1, Zip2, Zip3, Zip4, Spo16, Msh4, Msh5, and Mer3 (Börner et al., 2004). In Arabidopsis, mutations of Shoc1 (Zip2), Hei10, Zip4, Msh4, and Msh5 genes and double mutants result in an 80% to 90% reduction of CO number (Higgins et al., 2004, 2008b; Mercier et al., 2005; Chelysheva et al., 2007, 2012; Macaisne et al., 2011). It is thus clear that the ZMM pathway accounts for the majority of COs, but it is not the only pathway. Cytological data showed that numerous early recombination intermediates are labeled with ZMM proteins, such as ZIP4 and MER3, but only a few progressively mature into COs that are marked with HEI10 and MLH1 foci at the late pachytene stage (Lhuissier et al., 2007; Wang et al., 2009; Chelysheva et al., 2012; Shen et al., 2012). How these ZMM-dependent COs are designated remains to be investigated.

The class II COs do not exhibit interference and account for 10% to 20% of total COs (Berchowitz et al., 2007). Genetic analyses showed that MUS81 is required to generate class II COs, and its mutation leads to a reduction of COs by ~10%. However, a *msh4/mus81* double mutant of Arabidopsis still exhibited a mean of 0.8 chiasma per cell, suggesting that the formation of the class II COs can occur by other routes that have yet to be defined (Higgins et al., 2008a). It is also possible that blocking both class I and class II CO pathways triggers an aberrant type of CO formation that normally does not operate in the wild type. In *S. cerevisiae*, studies indicate that MUS81 acts as an endonuclease to resolve aberrant joint molecules that may be formed during the class I CO pathway (Hollingsworth and Brill, 2004). However, whether this is also the case for plants remains to be determined.

NCO Formation

In most organisms, there is a greater number of DSBs than COs, which may be needed to ensure sufficient interactions along the entire lengths of chromosomes. For example, maize meiosis generates ~500 DSBs and only ends with ~20 COs (Franklin et al., 1999). The DSBs that do not mature into COs are likely to form NCOs. These NCO recombination intermediates often first associate with heteroduplex DNA that contains both paternal and maternal strands. If any mismatched nucleotides are present in heteroduplex regions, they can be corrected to either version, leading to gene conversion events. Studies in *S. cerevisiae* suggested that NCOs can result from dissolution of the ssDNA invasion intermediates in a pathway called synthesis-dependent strand annealing (SDSA) (Pâques and Haber, 1999).

Recently, suppressor screens using Arabidopsis *zmm* mutants successfully led to the identification of three groups of genes that promote NCO formation. First, the helicase FANCM and its two cofactors, MHF1 and MHF2, are thought to unwind postinvasion intermediates to promote NCOs through the SDSA pathway (Crismani et al., 2012; Girard et al., 2014). In the Arabidopsis *fancm* mutant, CO frequency increases 3-fold compared with the wild type. These additional COs do not depend on ZMMs but require the MUS81 pathway, suggesting that, in the absence of FANCM, MUS81 repairs these intermediates (that normally FANCM acts on) and, as a result, generates extra class II COs. Second, the RTR complex, consisting of a RecQ helicase, a type IA topoisomerase, and the structural protein RMI1, is involved in the processing of DNA recombination intermediates in all eukaryotes (Mankouri and Hickson, 2007). In Arabidopsis, the *top3α-R640X* mutant, which introduces a premature stop codon at position 640, and the *recq4a recq4b* double mutation exhibit 1.5- and 6-fold increases in CO frequency, respectively (Séguéla-Arnaud et al., 2015). Genetic analyses showed that both TOP3α and RECQA/B function independently of FANCM helicase, suggesting that several pathways actively limit CO formation, presumably by processing different intermediate substrates. Third, the AAA-ATPase FIDGTIN-LIKE1 (FIGL1) also is an anti-CO protein that functions independently of FANCM (Girard et al., 2015). The observations of increased RAD51 foci and persistent DMC1 foci in the *figl1* mutant suggest that FIGL1 may control the dynamics of the two recombinases. Interestingly, the additional COs in these three groups of mutants are likely class II COs, because they depend on MUS81 and only show weak CO interference. Combining mutations in these genes results in a further additive increase in COs with a significantly elevated recombination rate at subtelomeric regions (see Box 1). Homologs of these antirecombination factors have been

Figure 1. (*Continued.*)
and DMC1, which may be regulated by FIGL1 (c). Strand invasions also can occur between sister chromatids (not shown). Second end capture, followed by DNA synthesis and ligation, results in the formation of a double Holliday junction (dHJ) that is protected by ZMM components (d and e). The dHJ can be resolved into class I COs that are subjected to interference (f). In contrast, NCOs are actively promoted by unwinding and resolving joint molecules. In the synthesis-dependent strand annealing (SDSA) pathway, DNA helicase disassembles the early strand invasion intermediates and the 3' overhang reanneals with the original double-strand duplex (g and h). Other intermediates can be matured into NCOs through different mechanisms (i). Some joint molecules and perhaps aberrant intermediates can be resolved as class II COs by MUS81 (k). These COs belonging to class II are insensitive to interference.
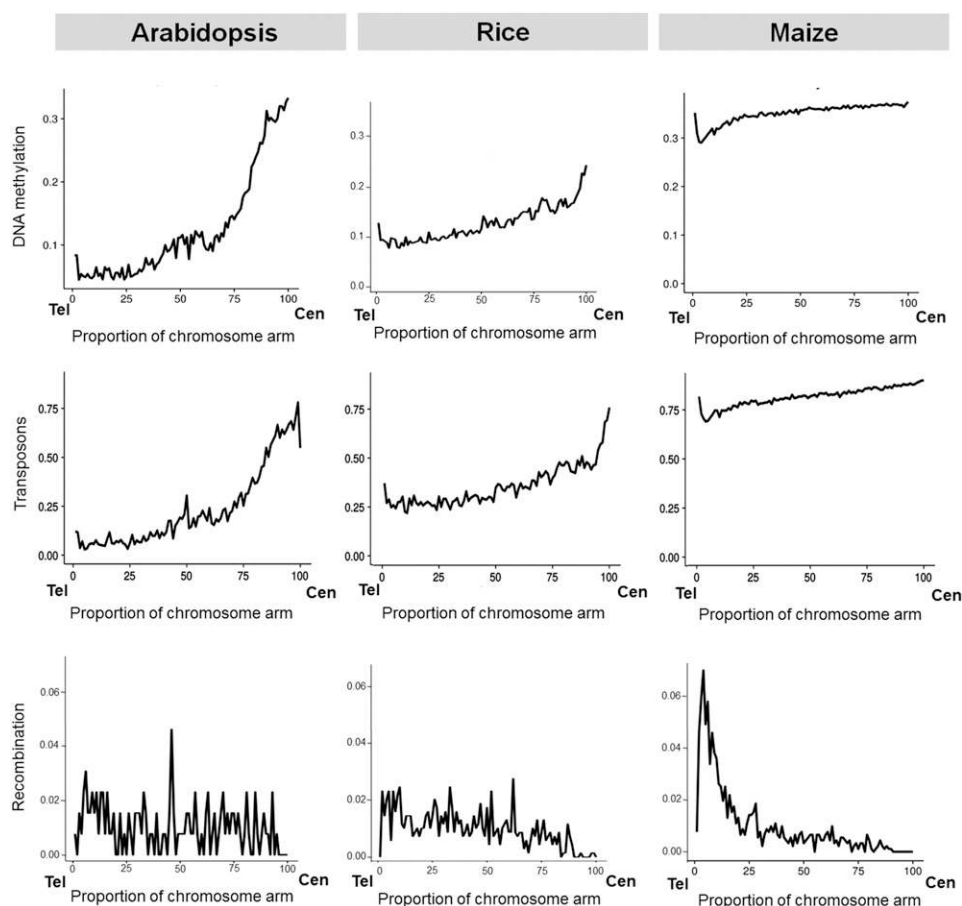
Figure 2. Genome structure and CO distribution differ between Arabidopsis, rice, and maize. Arabidopsis (Stroud et al., 2013; Wijnker et al., 2013) and rice (Chodavarapu et al., 2012; Si et al., 2015) have low DNA methylation and transposon content along chromosome arms. Both factors are high in the recombination-repressed heterochromatic regions close to centromeres. In contrast, maize has a much higher level of DNA methylation than Arabidopsis, and its chromosomes have a more homogenous composition of DNA methylation and transposons (Regulski et al., 2013; Li et al., 2015). The subtelomeric regions of maize have less DNA methylation and fewer transposons where meiotic recombination is mostly confined. Tel represents telomeres, and Cen represents centromeres.

identified in crops, but a phenotypic analysis of these genes has yet to be reported.

CO Distribution

The distribution of COs can be studied classically by generating a genetic map from the progeny of an F1 hybrid. Two markers physically close to one another on a chromosome will likely be coinherited. However, if a CO event occurs between these markers, it will break the genetic linkage and the markers will segregate independently. Thus, historical CO events can be deduced from linkage disequilibrium analysis that measures the association of sets of markers on chromosomes in a population. Recently, whole-genome sequencing of four products of meiotic tetrads allowed fine-scale mapping of recombination break points (Wijnker et al., 2013; Li et al., 2015). COs also can be scored cytologically by counting chiasmata or recombination nodules, seen as electron-dense structures at recombination sites by electron microscopy (Anderson et al., 2003). Furthermore, a powerful visual assay of fluorescent tetrad analysis has been used to measure regional CO rates in Arabidopsis (Francis et al., 2007).

COs are derived from the DSB events, but not every DSB has an equal probability of giving rise to a CO. Studies have shown that, as in other eukaryotes, the distribution of COs on plant chromosomes is not uniform but tends to be clustered in small regions called CO hotspots. Linkage analyses deduced from haplotype maps of diverse maize lines (Gore et al., 2009) and from an experimental F2 population of wheat (*Triticum aestivum*; Saintenac et al., 2009) revealed that CO frequency is higher in subtelomeric regions and lower in interstitial regions. In addition, CO formation is repressed at the centromeres and telomeres. This recombination landscape is markedly different from that of the model plant Arabidopsis, where CO hotspots are detected along the entire chromosomes, except at the centromeres, where recombination is repressed (Salomé et al., 2012). This difference may be attributable to diverse genome organizations. The Arabidopsis genome has a relatively low transposon content, and they are mostly located in the pericentromeric and centromeric regions where recombination is suppressed (Yelina et al., 2015; Fig. 2). In contrast, cereals with large genomes such as maize have many more transposons that are widely distributed on chromosome arms, with a slight decrease in small regions around the distal ends

(Regulski et al., 2013). COs are enriched in the distal gene-rich euchromatic regions (Fig. 2). These studies suggest a link between CO formation and chromatin structures.

In accordance with this latter supposition, analyses in rice, maize, and wheat at the level of megabase genomic windows suggest that CO hotspots identified from maize microscopes (Li et al., 2015) and F2 populations (Saintenac et al., 2011; Rodgers-Melnick et al., 2015; Si et al., 2015) have low DNA methylation and transposons but are enriched in regions with high gene density. At a smaller physical scale (kilobase windows), hotspots are polarized toward the 5' and 3' ends of genes (Xu et al., 1995; Li et al., 2015). This is similar to the recent finding in Arabidopsis that historical COs and recombination in male meiosis are associated with active chromatin features, such as H2A.Z, H3K4me3, low DNA methylation, and low-nucleosome-density regions (Yelina et al., 2012; Choi et al., 2013; Drouaud et al., 2013). These observations indicate that open chromatin structure is an important feature of CO hotspots in plants (see Box 1).

Synapsis and CO Designation

Meiotic recombination is concurrent with synapsis, a process during which homologous chromosomes are associated tightly with each other via the assembly of the SC (Page and Hawley, 2004). At early stages of recombination, when homologous regions are aligned, likely via DSB-dependent strand invasion, SC assembly occurs through loading of the transverse filament proteins between axial elements (AEs) of homologous chromosomes (Fig. 1B). After CO formation is complete, the SC is then disassembled, leaving CO sites to maintain the connection between homologs. Recombination, including DSB formation and CO maturation, occurs in the context of AEs and the developing SC. Studies have suggested an intimate yet unclear relationship between SC components and CO designation.

Three components of the AE have been identified in Arabidopsis and crops: (1) the meiotic cohesin subunit REC8 (Cai et al., 2003; Golubovskaya et al., 2006; Shao et al., 2011); (2) ASY1/PAIR2 (Armstrong et al., 2002; Nonomura et al., 2006; Boden et al., 2007), the functional homolog of Hop1; and (3) ASY3/PAIR3/DSY2 (Yuan et al., 2009; Ferdous et al., 2012; Lee et al., 2015), the functional homolog of Red1. In *S. cerevisiae*, the chromosome axis is essential for DSB formation (Mao-Draayer et al., 1996). Similarly, a reduction in DSB formation was reported in *asy3/pair3/dsy2* mutants. Moreover, the DSB-repair bias that favors interhomolog recombination is partially mediated by AE proteins (Sanchez-Moran et al., 2007; Kim et al., 2010). Despite the similarity of functions of axial proteins, a striking difference exists across plant species. In barley (*Hordeum vulgare*), maize, and wheat, chromosome axis formation and SC nucleation are initiated first in subtelomeric regions (Golubovskaya et al., 2011; Higgins

et al., 2012; Khoo et al., 2012). In contrast, axis and SC nucleation do not appear to be polarized in Arabidopsis and rice (Higgins et al., 2005; Wang et al., 2010). It is conceivable that the polarization of axis and SC formation toward telomeres, in combination with telomere clustering, helps the pairing of homologous chromosomes in species with large genomes (Fig. 1). Recent studies in barley showed that ~200 DSBs marked with γH2AX first appear in subtelomeric regions, which is coincident with the appearance of ASY1 (Higgins et al., 2012). γH2AX foci increased progressively and were distributed to interstitial regions of chromosomes. A similar spatiotemporal pattern of RAD51 localization also was observed in maize (Franklin et al., 1999). The subtelomeric regions with early recombination initiation of that first synapse correlate with subtelomeric CO preference, suggesting that the timing of recombination initiation also may influence CO formation.

Homologous engagement and SC formation trigger chromosome axis remodeling, as ASY1/PAIR2 is depleted from the lateral elements of the mature SC in a process dependent on the AAA-ATPase protein PCH2 (CRC1 in rice; Miao et al., 2013; Lambing et al., 2015). The reason for controlling the ASY1/PAIR2 association with the axis is unclear, but it is thought that ASY1/PAIR2 promotes interhomolog recombination and that removal of this protein from the synapsed regions, which nucleate at the CO designated sites, would allow repair of the remaining DSBs toward the sister chromatid or NCO (see Box 1). Phenotypic analyses of *pch2/crc1* mutants in Arabidopsis and rice revealed some differences. Rice CRC1 is required for ASY1/PAIR2 loading, but Arabidopsis PCH2 is responsible for ASY1 removal. Interestingly, an in vitro study suggests that *S. cerevisiae* Pch2 could promote both the association and dissociation of Hop1 (the ASY1/PAIR2 functional homolog) with DNA (Chen et al., 2014).

The function of the SC transverse filament ZYP1/ZEP1 on CO formation differs between Arabidopsis/barley and rice. In Arabidopsis and barley, downregulation of ZYP1 protein leads to a reduction in chiasma number and the presence of univalent chromosomes (Higgins et al., 2005; Barakate et al., 2014). In the rice *zep1* mutant, CO frequency and chiasma number are both increased while genetic CO interference is reduced (Wang et al., 2010). In addition, a recent report found an increased number of ZIP4 foci in the *zep1* mutant (Wang et al., 2015). It is possible that the absence of ZEP1 could impact axis compaction and, hence, alter the recombination events, leading to an increase in CO number. A similar increased CO rate in an SC-defective line was reported in other organisms. For example, in *S. cerevisiae* mutants devoid of the SC central element components Ecm11 and Gmc2, and in mutants expressing a version of Zip1 missing most of its N terminus, CO numbers also were increased (Voelkel-Meiman et al., 2016). However, it is not fully understood how the SC could influence the recombination rate.

PROGRESS IN MANUPULATING MEIOTIC RECOMBINATION

Abiotic and Biotic Treatments to Alter Recombination

Methods to increase COs in recombination-poor regions would increase genetic diversity and benefit plant breeders. Various abiotic and biotic treatments have been applied to alter CO distribution in crops. Meiotic recombination is influenced by environmental cues. For example, a change in temperature from 22°C to 30°C caused a reduction in chiasmata and seed set in barley. However, the distribution of COs was altered, and there were significantly more COs in the interstitial regions at the higher temperature (Higgins et al., 2012). In Arabidopsis, a progressive increase in temperature from 18°C to 28°C was associated with an increase in recombination frequency at two genetic intervals (Francis et al., 2007). Several lines of evidence suggest that the nutritional availability in the environment also impacts meiotic recombination. Increased potassium or phosphate contents enhance the recombination rates in barley, rye (*Secale cereale*), and rice (Law, 1963; Bennett and Rees, 1970; Fedak, 1973). It is thought that these elements alter the physiology of the cells and the conformation of the DNA. A recent study identified two quantitative trait loci for recombination in *Drosophila melanogaster*. One corresponded to a protein involved in the potassium channel and the other one is a protein with ATP-binding and transporter activities. This highlights the link between meiotic recombination and cellular physiological status (Hunter et al., 2016).

DNA-damaging agents have been used successfully to induce meiotic recombination in DSB mutants (Sanchez-Moran et al., 2007) and some wild-type plants (Lawrence, 1961). For example, cisplatin and UV exposure cause different forms of DNA damage that can be processed by the homologous recombination pathway and form COs during meiosis. In Arabidopsis, cisplatin can partially restore the number of bivalent chromosomes in the *spo11-1* mutant, in which programmed meiotic DSBs are abrogated. The drawback of using these approaches is that the ends of the DNA breaks are not covalently bound by SPO11, which is thought to inhibit the error-prone alternative end-joining pathway to repair the DNA damage (Lemmens et al., 2013). In Arabidopsis, it is not possible to create a sufficiently high number of cisplatin-generated DSBs that would allow normal pairing and COs without accumulating error-prone recombinations that cause genome instability (Sanchez-Moran et al., 2007). In contrast, a number of cases, such as that of *Lilium* spp., showed an increase in chiasma numbers in wild-type plants after irradiation (Lawrence, 1961).

Pathogen infection was shown to increase the somatic recombination rate in Arabidopsis (Kovalchuk et al., 2003). Cytological analysis in tomato (*Solanum lycopersicum*) and barley plants infected with viruses revealed that the positions of the chiasmata are shifted toward the interstitial regions (Andronic, 2012).

However, the total chiasma number was not increased significantly, although aberrant tetrads were detected. Interestingly, recent fine-scale mapping of COs in rice showed that genes located at recombination hotspots are involved in responses to environmental stimuli. That study also showed that heat stress and pathogen infection increased the recombination rate for some individuals (Si et al., 2015).

NEGOTIATING MEIOSIS IN POLYPLOIDS

In contrast to animals, which have mainly diploid genomes, plants are frequently polyploid, possessing upwards of two sets of chromosomes. This phenomenon creates a particular challenge to ensure accurate chromosome segregation in meiosis. Moreover, since many crop species are polyploid, it is an issue that has to be considered when attempting to modulate CO formation. Allopolyploids arise through the hybridization of genomes from species with closely related, yet distinct, homologous chromosomes. Genome doubling within a species gives rise to autopolyploids, which possess multiple copies of identical homologous chromosomes. Despite these different origins, the meiotic imperative is the same (i.e. to behave like diploids during meiosis). Establishing how this is achieved at the mechanistic level still has some considerable way to go, although significant progress has been made in some cases. As space is limited, this topic will not be discussed extensively here; however, there are several excellent reviews that discuss polyploid meiosis (Cifuentes et al., 2010; Grandont et al., 2013; Mercier et al., 2015; Lloyd and Bomblies, 2016).

Meiosis in Allopolyploid Species

Studies reveal that the diploid-like behavior of polyploid meiosis is achieved by preferential pairing of homologous chromosomes rather than random association between pairs of homologs. Although structural variation between homologs may favor this behavior, studies thus far suggest that additional genetic controls function to confine interactions to homologous chromosomes. Genetic loci have been identified in a number of species, including commercially important crops such as cotton (*Gossypium hirsutum*), wheat, and oilseed rape/canola (*Brassica napus*). Reflecting its importance as a crop, allohexaploid bread wheat (AABBDD; $2n = 6x = 42$) has been studied most extensively. Several loci controlling pairing behavior exist in wheat, among which *Pairing homologous1* (*Ph1*), found on chromosome 5B, has the greatest influence and is the most studied (Riley and Chapman, 1958; Sears, 1976). Deletion lines lacking *Ph1* exhibit extensive multivalent formation at metaphase I arising from COs between homologs, which lead to defects in chromosome segregation resulting in reduced fertility. This was reported to occur through the influence of *Ph1* on a range of processes

required for meiotic progression from S phase through metaphase I. These include the rate of premeiotic replication, coordination of homolog pairing through chromatin remodeling, resolution of interlocked chromosomes in zygotene, and disrupted chromosome alignment at metaphase I (Greer et al., 2012). Molecular analysis of the *Ph1* locus has revealed the presence of a cluster of *CYCLIN DEPENDENT KINASE*-like (*CDK*-like) genes with similarity to the human cell cycle *cdc2* gene (Al-Kaff et al., 2008). The CDK-like cluster on chromosome 5B, which is associated with the dominant *Ph1* phenotype, is disrupted by the presence of a segment of heterochromatin. Although the 5B *CDK* genes are transcribed, they are defective and are thought to suppress the expression of the corresponding regions on the other homologs (Al-Kaff et al., 2008). Deletion of *Ph1* is proposed to lift suppression, leading to increased CDK activity and resultant meiotic defects. The mechanistic basis for this outcome has yet to be established. However, studies reveal a link with two proteins (ASY1 and MLH1) important in chromosome alignment, synapsis, and recombination. Expression of the protein axis component ASY1 is increased in a *ph1* mutant (Boden et al., 2007, 2009). An elevated level of ASY1 might perturb normal synapsis, as its depletion from synapsed regions of the axis is required for polymerization of the SC. This is a critical step in meiotic progression, and it is interesting that another locus on chromosome 3D, *Ph2*, which stabilizes diploid-like meiotic behavior in wheat, is required for the progression of synapsis (Ji and Langridge, 1994). *Ph1* also has been found to prevent CO maturation of MLH1-marked recombination intermediates on associated homologs (Martín et al., 2014). Furthermore, MLH1 is associated with a role in the removal of chromosomal interlocks (Storlazzi et al., 2010). As multivalents arising from COs between homologous chromosomes and persistent interlocks are characteristics of a *Ph1* deletion, it is plausible that MLH1 might be a component of the *Ph1* regulatory network.

In addition to the CDK-like cluster, another gene, *C-Ph1*, has been identified at the *Ph1* locus, adding further complexity (Bhullar et al., 2014). Functional analysis of a putative Arabidopsis ortholog of *C-Ph1* using RNA interference resulted in a phenotype reminiscent of the wheat *ph1* mutant. Further studies will be required to resolve the exact contribution of the 5B CDK-like cluster and *C-Ph1* in meiotic diploidization of wheat meiosis.

A second suppressor of homologous chromosome pairing in wheat, *Ph2*, also has been identified, although it is less effective than *Ph1*. The *Ph2* locus encodes numerous genes, among which is the DNA mismatch-repair gene *TaMSH7*, which is considered a promising candidate for *Ph2* (Mello-Sampayo, 1971; Sutton et al., 2003).

Canola (AACC; $2n = 28$) is an allotetraploid crop plant that behaves as a diploid during meiosis. It arises from the hybridization of *Brassica rapa* (AA; $2n = 20$) and *Brassica oleracea* (CC; $2n = 18$). It is estimated to have arisen ~7,500 years ago, but it has been resynthesized on numerous occasions. Generally, COs are restricted

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OUTSTANDING QUESTIONS

- The distribution of DSB hotspots and their relationships to epigenetic markers and chromosome axes in plants remain to be discovered.

- The molecular machinery of DSB formation needs to be deciphered, such as the absolute requirement for break formation and spatiotemporal control of DSB activity.

- What factors suppress proximal COs and control CO frequency in plants? Are the timing of DSB formation, chromatin structures, remodeling of chromosome axes or dynamics of SC involved in CO designation, and how?

- What is the mechanistic basis of temperature-regulated CO formation? Do any other biotic or abiotic treatments impact CO positioning?

- Several mutants affecting CO frequency and positioning have been identified in Arabidopsis, but how can such knowledge be exploited to manipulate recombination in crops?

- By which mechanism do polyploid plants ensure diploid-like chromosome pairing?

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to homologous chromosomes, although recombination between homologs is observed (Grandont et al., 2014). Insight into CO control in canola has come from the analysis of AC genome allohaploids possessing 19 chromosomes. Two groups of allohaploids can be identified with either low or high levels of homologous recombination. Analysis of a segregating population derived from crossing a member of each type has led to the identification of several quantitative trait loci affecting homologous recombination. Of these, the major determinant, termed *Pairing regulator in Brassica napus*, has been mapped to chromosome C_9 (Jenczewski et al., 2003).

Enhanced CO Formation in Allotriploid *Brassica* spp.

Taking a lead from research in *Caenorhabditis elegans* and earlier work in plants suggesting that the presence of unpaired chromosomes could lead to an increase of COs between the chromosomes engaged in bivalent formation, recent studies have demonstrated this effect in *Brassica* spp. allotriploids. Initially, triploid lines

(AAC) comprising a diploid *Brassica nigra* (AA) genome and a haploid *B. oleracea* (C) genome were found to exhibit increased CO formation of 1.7-fold the diploid (AA) level. The fertile AAC hybrids showed the formation of bivalents between A chromosomes, the C chromosomes remaining unpaired as univalents. The increase of CO formation in the AAC triploid hybrid was accompanied by an increase in the number of MLH1 foci at diakinesis, implying that, at least in part, this was due to an elevation of class I interference-sensitive COs. The study also revealed that the allotetraploid (AACC) displayed an increase in COs to a level intermediate between the diploid (AA) and allotriploid (AAC) hybrid (Leflon et al., 2010). Even more remarkably, in a subsequent study, the same workers examined hybrids containing the diploid A genome combined with different numbers or specific individual C chromosomes. They observed increases of up to 4.1-fold of the recombination rate in the 9C hybrid (AA plus nine additional C chromosomes). When plants carrying one extra C_9 chromosome (AA + $1C_9$) were examined, the recombination rate was 2.7-fold higher than that of the AA diploid genome. This finding suggests that the number of unpaired C chromosomes and the presence of specific C chromosomes in the hybrids modulated the frequency of COs on the AA genome (Leflon et al., 2010; Suay et al., 2014).

Autopolyploid Meiosis

Autopolyploids possess duplicate sets of chromosomes. Hence, for each homologous chromosome, a number of cognate pairing partners are available depending on the ploidy level. A study of Arabidopsis neoautotetraploids revealed extensive multivalent formation at metaphase I, leading to errors in chromosome segregation and subsequent reduced fertility. However, partial diploidization became apparent within a few generations. This appeared to be achieved through a reduction in CO frequency as fewer chiasma were observed at metaphase I, leading to a reduction in multivalents and a corresponding increase in bivalents (Santos et al., 2003). However, in other studies, Arabidopsis and maize autotetraploids have been reported to exhibit increased levels of recombination (Bingham et al., 1968; Pecinka et al., 2011). The basis of these different results is unresolved thus far and will require additional analysis. Recent studies in natural populations of *Arabidopsis arenosa* that occur in both diploid and autotetraploid forms have provided further clues to how meiosis can become stabilized in autopolyploids (Lloyd and Bomblies, 2016). Cytological analysis of *A. arenosa* tetraploids reveals that the sets of homologous chromosomes predominantly form two pairs of bivalents rather than multivalents. This outcome contrasts with neoautotetraploids derived from diploid *A. arenosa* plants and is associated with a reduction in chiasma frequency (Yant et al., 2013). Notably, despite the reduced CO frequency, univalent chromosomes at metaphase I are rare, suggesting that CO control is maintained. It is hypothesized that a reduction in CO frequency, which favors bivalent formation, is achieved through an increase in CO interference rather than as a direct effect on the recombination pathway (Bomblies et al., 2016). The rationale for this hypothesis is that the former would ensure an obligate CO between each pair of homologs while the latter would not ensure this; hence, COs would be lost at random, thereby increasing the risk of univalents at metaphase I. How CO interference is mediated remains to be resolved (see above), but recent studies implicate the chromosome axis. Consistent with the interference hypothesis, comparative genome sequencing of tetraploid and diploid *A. arenosa* has revealed strong selection within the tetraploid lineage of a number of genes encoding key axis components (ASY1, ASY3, and SYN1/REC8) and associated proteins (ZYP1A/B and PDS5; Yant et al., 2013).

CONCLUSION

In recent years, studies from Arabidopsis have identified about 80 meiotic genes, with most of them involved in recombination, and have gained a better understanding of the mechanisms and controls of meiotic DSB and CO formation in plants. Yet, much remains to be learned, with current knowledge providing basic frameworks for exploring strategies to manipulate meiotic recombination in crops. Cereal genomes have more repetitive sequences, are much larger than the Arabidopsis genome, and often even contain duplicated chromosomal segments due to ancient polyploidization, so different aspects such as the dynamics of gigantic chromosomes during pairing, functional specialization after gene duplication, and mechanisms to avoid homologous pairing should be taken into account.

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