Molecules and Cells

Minireview

Fast and Precise: How to Measure Meiotic Crossovers in *Arabidopsis*

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During meiosis, homologous chromosomes (homologs) pair and undergo genetic recombination via assembly and disassembly of the synaptonemal complex. Meiotic recombination is initiated by excess formation of DNA double-strand breaks (DSBs), among which a subset are repaired by reciprocal genetic exchange, called crossovers (COs). COs generate genetic variations across generations, profoundly affecting genetic diversity and breeding. At least one CO between homologs is essential for the first meiotic chromosome segregation, but generally only one and fewer than three inter-homolog COs occur in plants. CO frequency and distribution are biased along chromosomes, suppressed in centromeres, and controlled by pro-CO, anti-CO, and epigenetic factors. Accurate and high-throughput detection of COs is important for our understanding of CO formation and chromosome behavior. Here, we review advanced approaches that enable precise measurement of the location, frequency, and genomic landscapes of COs in plants, with a focus on *Arabidopsis thaliana*.

Keywords: crossover, fluorescence-tagged lines, genotyping-by-sequencing, interference, meiosis, synaptonemal complex

INTRODUCTION

Meiosis refers to specialized cell division in sexually reproducing eukaryotes (Villeneuve and Hillers, 2001). The process involves a single round of DNA replication and two successive rounds of cell division, with the resulting cells having half the number of chromosomes as the parent cell. During meiosis in most diploid eukaryotes, homologous chromosomes (homologs) pair to form bivalents and undergo reciprocal exchange of genetic material, called crossover (CO). The presence of at least one CO per bivalent is essential for the accurate segregation of homologs and ensures the generation of viable gametes because the absence of CO results in unbalanced chromosome segregation at meiosis I and aneuploid cells. COs also contribute to genetic diversity in populations, which facilitates local adaptation and breeding in animals and plants (Barton and Charlesworth, 1998).

Meiotic COs are formed by the repair of DNA double-strand breaks (DSBs) induced by topoisomerase-like SPO11 and its associated proteins (Kim and Choi, 2019; Lam and Keeney, 2014). The progression of meiotic recombination is tightly connected to the dynamics of chromosome behavior, including chromosome axis-loop formation, homolog alignment, and synaptonemal complex (SC) assembly and disassembly (Fig. 1A) (Ur and Corbett, 2021; Zickler and Kleckner, 1999). At DSB sites, the 5′ end is bidirectionally resected to produce a 3′ single-strand DNA. Subsequently, the 3′ end undergoes a search for homologs or sister chromatids with the assistance of recombinases such as DMC1 and/or RAD51. The inter-homolog invasion forms a recombination intermediate, called a displacement (D) loop or joint molecule. DNA synthesis extends the D-loop to generate a double Holliday junction (dHJ) intermediate that is resolved to generate CO or non-CO products. COs are formed by two con-

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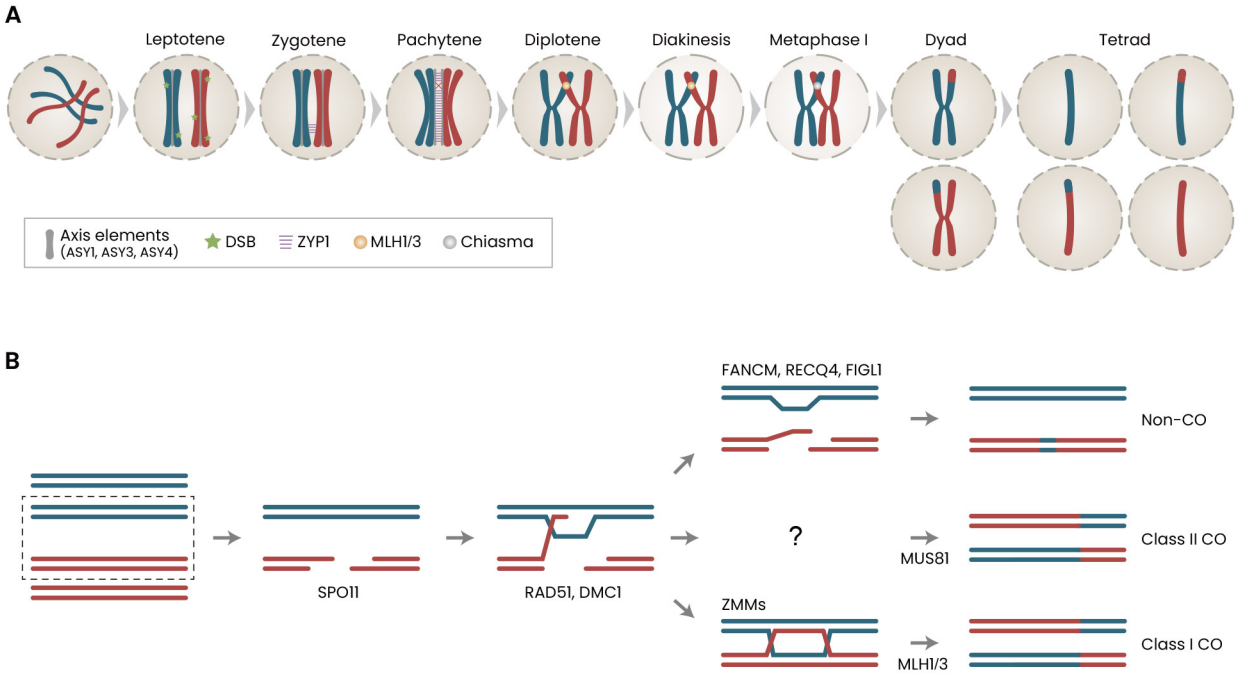

Fig. 1. Dynamics of meiotic chromosomes and CO during meiosis. (A) Changes in meiotic chromosomes for recombination during meiosis. At the leptotene stage, chromosomes form axis elements and DSBs are generated in excess. A subset of DSBs are repaired as COs. In the zygotene stage, assembly of the SC is initiated at designated CO sites. In the pachytene stage, SC assembly is complete. ZMM MSH4/5, HEI10 and MLH1/3 localize to CO sites. In the diplotene stage, SC disassembles and homologs remain linked by COs. In the diakinesis stage, chromosomes condense and COs can be detected by acid spreading. In metaphase I, chromosomes are highly compacted and bivalents align at the metaphase I plate, linked by COs called as chiasmata. (B) Model of meiotic recombination. SPO11 and its accessory proteins catalyze the formation of meiotic DSBs. DSB ends are resected to produce 3′ single-strand tails which, with the help of RAD51 and DMC1, invade non-sister chromatids to form D-loops. The majority of D-loops are resolved into non-COs while approximately 5% of D-loops mature into COs via two pathways. Class I COs are interference sensitive and depend on ZMMs and MLH1/3, representing approximately 85%-90% of total COs. Class II COs are non-interfering, formed by MUS81, and restricted by anti-CO factors such as FANCM.

served CO pathways in most eukaryotes, named class I and class II (Fig. 1B) (Mercier et al., 2015). In most plants, the class I pathway depends on a group of pro-CO proteins, called ZMMs (ZIP4, MSH4, MSH5, MER3, HEI10, PTD, SHOC1) and MLH1/MLH3 heterodimeric endonucleases (Mercier et al., 2015). Class I COs account for approximately 80%-85% of COs in plants and are sensitive to CO interference. The remaining 10%-15% of COs are interference-insensitive and depend on MUS81 in the class II pathway. Class II COs are restricted by anti-CO factors that promote the generation of non-COs, such as FANCM (Crismani et al., 2012; Mercier et al., 2015; Séguéla-Arnaud et al., 2015; Taagen et al., 2020).

CO frequency and distribution are tightly regulated, which is manifested in phenomena such as CO homeostasis, assurance, and interference. However, the underlying mechanisms remain elusive. COs are homeostatically controlled in many organisms, maintaining consistent CO frequencies despite variations in the number of DSBs in yeast, mice, and the nematode *Caenorhabditis elegans* (Cole et al., 2012; Martini et al., 2006; Rosu et al., 2011; Yokoo et al., 2012). However, COs are somewhat less strictly controlled in plants (Sidhu et al., 2015; Xue et al., 2018). CO interference refers to non-random spacing of COs along the chromosome, with the occurrence of one CO inhibiting the formation of adjacent COs (Zickler and Kleckner, 1999). The occurrence of more than two COs per pair of homologs is rare among most species (Fernandes et al., 2018) and CO patterns not following Poisson distribution show the effect of interference (Berchowitz and Copenhaver, 2010). Specifically, COs are favored in narrow regions of approximately 1-2 kb at gene promoters, terminators and specific DNA transposons in plants, called recombination hotspots, where nucleosome density and DNA methylation levels are low (Choi, 2017; Choi and Henderson, 2015).

Precise and high-throughput measurements of COs are important for understanding the mechanisms that control meiotic recombination. Cytological analyses, immunostaining, segregation assays of genetic markers, next-generation sequencing, and long-read sequencing methods have been extensively developed to measure CO patterns (Fig. 2, Tables 1 and 2). Here, we provide an overview of the methods used to visualize and detect CO events in *Arabidopsis thaliana* and highlight novel techniques.

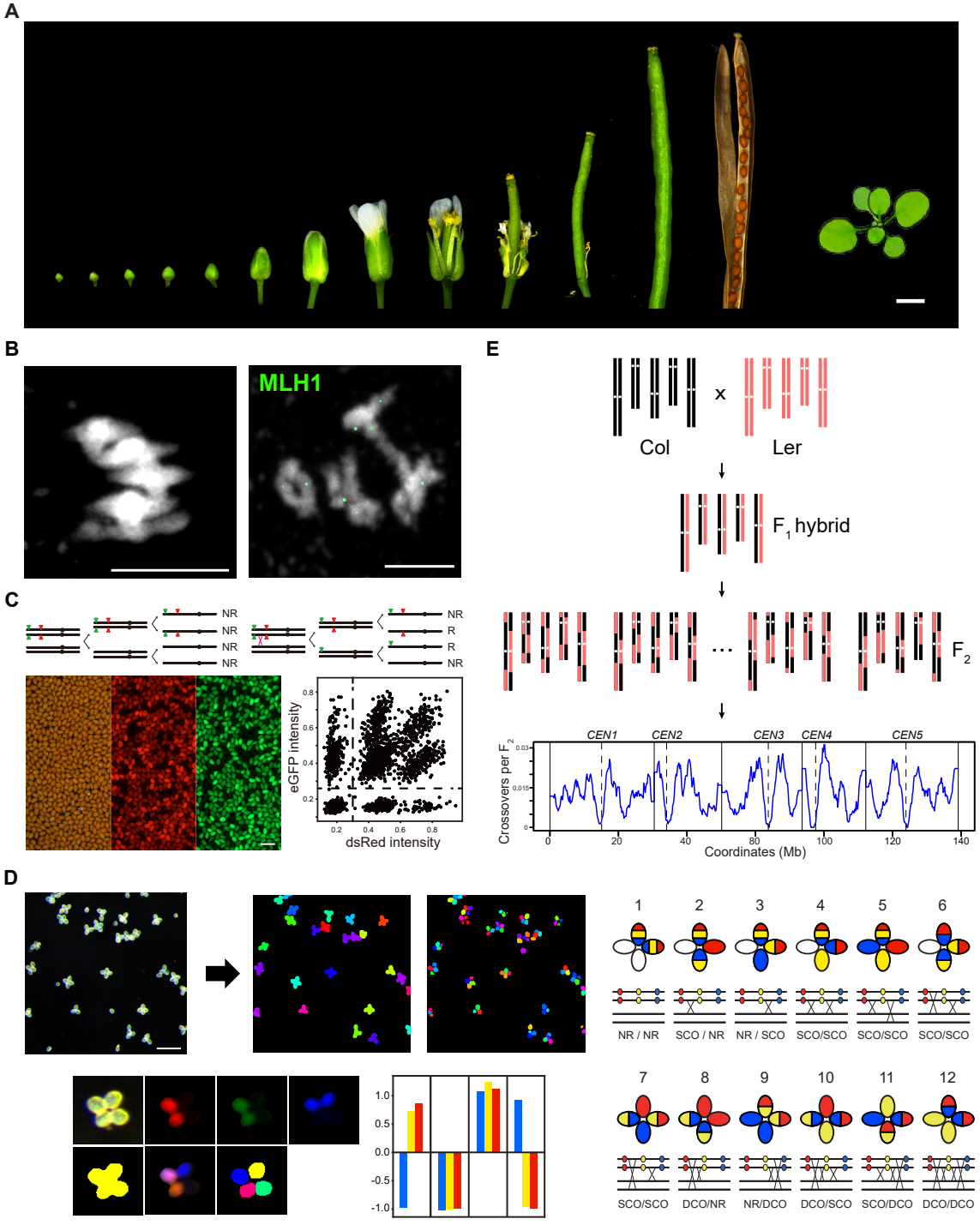

Fig. 2. Approaches to detect COs. (A) *Arabidopsis* developmental stages for analyzing meiotic recombination. Closed buds (0.3-0.5 mm in size) are used for cytological analysis. Mature pollen and seeds are analyzed using pollen and seed FTLs, respectively. Leaves are analyzed by GBS. Scale bar = 1 mm. (B) Cytological analysis of COs. DAPI staining (white) of chromosomes at metaphase I (left image). MLH1 (green) immunostaining at the diakinesis stage (right image). Scale bars = 0.5 μm. (C) Seed FTLs. Segregation of fluorescent reporters is shown during meiosis as non-recombinants and recombinants. NR, non-recombinant; R, recombinant. Fluorescent proteins in seeds from self-fertilized hemizygous plants (*GR/++*) that contain two T-DNAs (GFP, RFP) on the same chromosome are analyzed by CellProfiler. Scale bar = 1 mm. (D) Three-color pollen FTLs in *qrt* mutant background. DeepTetrad software accurately analyzes images of fluorescent pollen tetrads in a high throughput manner. Scale bar = 0.25 μm. Pollen FTLs produce 12 types of tetrads according to the location and number of CO. NR, non-recombinant; SCO, single crossover; DCO, double crossover. (E) Genome-wide CO map by GBS. Two different accessions (Col and Ler) are crossed and F_1 plants are self-fertilized. Sequencing and GBS libraries are constructed for F_2 individuals to precisely map COs on the genome.

Table 1. Comparison of CO measurement methods

		Material	Equipment	Time for preparation	Time for data analysis	CO interference measurement	Single-interval DCO measurement	High-throughput analysis	References
Cytology	Chiasmata		FM	1 day	1 h	No	No	No	Armstrong, 2013 Kurzbauer et al., 2018 López et al., 2012 Sanchez-Moran et al., 2002
	MLH1 foci	MLH1 antibody	CLSM	~2 days	1 h	No	No	No	Chelysheva et al., 2010 Lloyd et al., 2018
Seed-based		Seed FTLs	FM, CellProfiler	1 h	1 h	No	No	Yes	Melamed-Bessudo et al., 2005 Wu et al., 2015
Pollen-based	Manual counting	Pollen FTLs	FM, graphics software	2.5 h	1 day	Yes	Yes	No	Berchowitz and Copenhaver, 2008 Fernandes et al., 2018 Francis et al., 2007
	FACS	Pollen FTLs	FM, flow cytometer	1 h	5 h	Yes	No	Yes	Yelina et al., 2013 Ziolkowski et al., 2017
	DeepTetrad	Pollen FTLs	FM, DeepTetrad package	1 h	2.5 h	Yes	Yes	Yes	Lim et al., 2020 Nageswaran et al., 2021
GBS		F_2 hybrid population GBS library		2 days	1 month	Yes	No	No	Nageswaran et al., 2021 Rowan et al., 2015

CO, crossover; DCO, double crossovers; FTLs, fluorescence-tagged lines; FM, fluorescence microscope; CLSM, confocal laser scanning microscopy; FACS, fluorescence-activated cell sorting; GBS, genotyping-by-sequencing.

Table 2. Strengths and weaknesses of CO measurement methods

		Strength	Weakness
Cytology	Chiasmata	• Quick and simple method to analyze CO numbers per cell	• Difficult to analyze large number of cells • Difficult to measure CO positon and frequency precisely
	MLH1 foci	• Visualize class I crossover sites per cell and per chromosome • Able to combine with other cytological analysis	
Seed-based FTLs		• High-throughput analysis of CO frequency is possible • Able to get the average of female and male-specific CO frequency	• CO rate measurement range is limited to 50 • Cannot detect DCOs • Fluorescence can be silenced or unstable
Pollen-based FTLs	Manual counting	• Able to detect DCOs and measure CO interference • No need to install graphic card and DeepTetrad or flow cytometer equipment	• Silencing of fluorescence can occur in some genetic backgrounds • Laborious
	FACS	• High-throughput analysis is possible	• Cannot measure double CO in a single interval • Requires flow cytometer equipment
	DeepTetrad	• Simple sample preparation • High-throughput analysis is possible	• Requires DeepTetrad pipeline
GBS		• Precisely detect genome-wide CO sites	• High-cost and time-consuming

CO, crossover; DCO, double crossovers; FTLs, fluorescence-tagged lines; FACS, fluorescence-activated cell sorting; GBS, genotyping-by-sequencing.

VISULALIZATION AND QUANTIFICATION OF CROSSOVERS

Cytological analysis is a powerful tool for evaluating meiotic chromosome behavior and COs (Fig. 1) (Sims et al., 2021). In *Arabidopsis*, pollen mother cells (PMCs) are surrounded by multiple layers of cells in the anther and enclosed in thick callose walls. Acid (ethanol-acetic acid) and detergent-nuclei spreading techniques have been extensively used as fixative and fresh sample preparation methods in the cytological analysis of COs. The acid spreading method enables the preparation of chromosomes encompassing all meiotic stages. Ethanol-acetic acid is used as a fixative for buds, enzymatic digestion, and ethanol-acetic acid spreading steps (Armstrong et al., 2001; Ross et al., 1996). Additional heat or microwave treatments increase the accessibility of epitopes to antibodies for immunostaining (Chelysheva et al., 2010). Meanwhile, the detergent spreading method was first developed for use in electron microscopy (Albini et al., 1984) and modified over time (Armstrong et al., 2002; Chelysheva et al., 2005). This method involves enzymatic pretreatment to weaken PMC walls, chromatin spreading with detergent (Lipsol), and fixation with formaldehyde. The fixed preparations on glass slides are then labeled with antibodies to quantify meiotic proteins. Since meiocytes comprise only a small portion of cells in the anther, the spread also contains non-meiotic cell debris that hinders the search for meiotic chromosomes. Pre-isolation of pure meiocytes by dissecting the anther concentrates meiotic nuclei and reduces background interference (Chen et al., 2010; Kurzbauer et al., 2012). Nevertheless, acid and detergent spreading methods distort the original structure of the nucleus by disrupting the three-dimensional (3D) configuration into a plane. Thus, a method to preserve nuclear integrity has been developed to gain a deeper understanding of chromosome behavior and meiotic protein dynamics (Hurel et al., 2018; Sims et al., 2020a).

Counting chiasmata

COs between homologous pairs in *Arabidopsis* have been visualized and scored as chiasmata at metaphase I using a simple combination of acid spreading, DAPI (4,6-diamidino-2-phenylindole) staining, and epifluorescence (Fig. 2A) (López et al., 2012; Moran et al., 2001; Sanchez-Moran et al., 2002). Bivalents are highly compacted at metaphase I and aligned at the metaphase I plate (Figs. 2A and 2B). However, meiotic recombination-defective mutants in *Arabidopsis*, such as *spo11* and *zip4* mutants, generate 10 univalents at metaphase 1 compared to the five bivalents found in wild-type plants. This method was used to successfully screen meiotic recombination mutants from sterile mutants (De Muyt et al., 2009). The bivalent configurations at metaphase I are classified as rods and rings. A single chiasma in only one

chromosome arm leads to a rod-shaped bivalent, whereas a ring-shaped bivalent has both chromosome arms bound by two or three chiasmata. Fluorescence *in situ* hybridization (FISH), also known as chromosome painting, uses fluorescent-labeled chromosome-specific nucleic acid probes to identify chromosomes and their aberrations (Cremer et al., 1988; Pinkel et al., 1988). In *Arabidopsis*, FISH staining of 5S and 45S rDNA regions enables the identification of individual chromosomes, and the number of chiasmata can be counted based on bivalent shape (López et al., 2012; Moran et al., 2001; Sanchez-Moran et al., 2002). Painting whole chromosomes by FISH has led to the investigation of meiotic pairing, chromosome rearrangements, and even CO sites in crop species (Albert et al., 2019; Zhao et al., 2019). For example, the CO sites in hybrid maize were visualized using a haplotype-specific FISH technique on metaphase chromosomes (do Vale Martins et al., 2019). FISH can also be combined with immunostaining to measure both the quantity and spatial distribution of meiotic proteins along chromosomes (Sims et al., 2020b).

Immunostaining of CO sites

Class I interfering COs have been visualized and quantified by immunostaining in *Arabidopsis* (Sims et al., 2021). MLH1 and MLH3 proteins localize at interfering CO sites from the late pachytene to diakinesis stages (Chelysheva et al., 2010). Acid spreading is often used with MLH1/3 immunostaining to determine the number of class I COs at the diakinesis stage (Fig. 2B) (Modliszewski et al., 2018; Nageswaran et al., 2021; Ziolkowski et al., 2017). Combining the detergent spreading method with confocal laser scanning microscopy (CLSM), MLH1/MLH3 proteins can be co-immunostained with REC8 or ZYP1 to visualize the fully synapsed chromosome axes or SC at the pachytene stage, thus enabling the number of class I COs to be counted (Capilla-Pérez et al., 2021; France et al., 2021; Lloyd et al., 2018). HEI10 protein, a conserved meiotic E3 ligase, promotes the number of class I interfering COs in a dose-dependent manner (Serra et al., 2018a; Ziolkowski et al., 2017). Abundant immunostained HEI10 foci (approximately 100-150) are visible in the early pachytene stage, but their numbers are gradually reduced to approximately 9-11 and overlap with MLH1 foci at the late pachytene stage (Chelysheva et al., 2012). Thus, co-immunostaining of HEI10 and ZYP1 at the late pachytene stage can help determine the number of COs. Recently, 3D-structured illumination microscopy (SIM) was used to measure the precise location, intensity, and number of immunostained HEI10 foci along the synapsed chromosome, which provided insight into predicting CO interference strength by HEI10 dosage and gradual coarsening (Morgan and Wegel, 2020; Morgan et al., 2021). Super-resolution microscopy techniques, such as SIM and stimulated emission depletion (STED) microscopy, have been recently applied to visualize meiotic proteins, chromosome axes, and SC in wild-type *Arabidopsis* and meiotic mutants (Capilla-Pérez et al., 2021; France et al., 2021; Morgan et al., 2021; Sims et al., 2021). These methods will help reveal the molecular mechanisms of CO formation and distribution.

HIGH-THROUGHPUT MEASUREMENT OF CROSSOVER FREQUENCY

In plants, COs can be detected using segregation assays that measure co-inheritance or separation of linked, heterozygous genetic markers on homologs during meiosis. Meiotic COs between markers lead to changes in the types of linkage in post-meiotic products such as pollen, seeds, and F_2 individuals. Single nucleotide polymorphisms (SNPs), simple sequence length polymorphisms (SSLPs), and transfer DNA (T-DNA) have been used as genetic markers (Copenhaver et al., 1998; Giraut et al., 2011; Salomé et al., 2012). Segregation assays with T-DNAs that express fluorescent proteins in seeds or pollen have been extensively developed to detect CO frequency in a high-throughput manner (Figs. 2C and 2D) (Berchowitz and Copenhaver, 2008; Francis et al., 2007; Melamed-Bessudo et al., 2005). Genome-wide sets of seed and pollen fluorescence-tagged lines (FTLs) are available, which facilitates the detection of CO frequency along chromosomes as well as in a specific region on a chromosome (Berchowitz and Copenhaver, 2008; Wu et al., 2015).

Seed FTL system

The seed FTL system uses T-DNAs expressing eGFP or dsRed in the seed-coat under a seed-specific napin promoter to measure CO frequency (Melamed-Bessudo et al., 2005; Wu et al., 2015). Each seed FTL contains a pair of homozygous T-DNA markers expressing eGFP (G) and dsRed (R) linked *in cis* on the same chromosome. Homozygous seed FTLs (*GR/GR, GFP RFP/GFP RFP*) are crossed with wild-type plants (*++/++*) to produce hemizygous plants (*GR/++*). Fluorescent proteins in seeds from individual self-fertilized plants (*GR/++*) can be visualized using different filters under a fluorescent microscope, thus distinguishing four types of seeds with green fluorescent protein (GFP), red fluorescent protein (RFP), no fluorescence, or both GFP and RFP markers (Fig. 2C). Images of 1,000-3,000 seeds per plant can be analyzed by CellProfiler, an image processing program that enables high-throughput measurement of sex-averaged CO frequency (Nageswaran et al., 2021; Ziolkowski et al., 2015; 2017). The seed FTL system has five advantages: (1) it provides a sufficient number of seeds for each individual plant, resulting in robust CO frequency between individuals of the same genotype with little variation; (2) seeds can be stored for future large scale analysis; (3) hemizygous seeds (*FTL GR/++*) can be pre-selected, saving time and space for plant growth; (4) a genome-wide set of seed FTLs is available for a landscape of CO frequencies along chromosomes; (5) sex-specific and average CO frequency can be measured (Saini et al., 2020). As a weakness the fluorescence of FTLs can be silenced or unstable across generations or in different genetic backgrounds, therefore the segregation ratio and intensity of FTLs should be carefully checked for CO measurement (Tables 1 and 2).

A sensitive seed FTL, the *420* line in Col-0 *Arabidopsis* accession where T-DNAs are located at the subtelomeric region of chromosome 3 (GFP-Chr3:256516 RFP-Chr3:5361637), has been extensively used to measure CO frequency (Melamed-Bessudo et al., 2005). Specifically, the *420* line was used to assess the reshaping of CO frequency patterns

along chromosomes due to DNA methylation deficiency, axis formation, and SC mutants, as well as the positive roles of H2A.Z deposition and regional heterozygosity in CO formation (Choi et al., 2013; France et al., 2021; Lambing et al., 2020; Yelina et al., 2012; Ziolkowski et al., 2015). Additionally, use of the *420* line led to the identification of *HEI10, TAF4B*, and *SNI1* as natural modifiers of CO frequency with different *Arabidopsis* accessions (Lawrence et al., 2019; Zhu et al., 2021; Ziolkowski et al., 2017). Moreover, the *420* seed FTL high-throughput system enabled the forward genetic screening of *low* or *high crossover rate* (*lcr* or *hcr*) mutants using ethyl methanesulfonate mutagenesis (Kim et al., 2021; Nageswaran et al., 2021). The seed FTL-based genetic screen revealed that *HCR1* encodes a PPX1 phosphatase that limits class I COs by interacting with ZMM proteins, and that HCR2/HSBP is abundantly expressed in meiocytes and restricts the number of COs by repressing *HEI10* transcription via attenuation of heat shock factor activity (Kim et al., 2021; Nageswaran et al., 2021).

Pollen FTL system

The pollen FTL system provides a powerful tool for the detection of COs and tetrad analysis in *Arabidopsis* male meiosis (Fig. 2D) (Berchowitz and Copenhaver, 2008; Francis et al., 2007). Pollen FTLs contain T-DNAs expressing eCFP, dsRed, or eYFP fluorescent proteins in mature pollen under the post-meiotic promoter *LAT52*. First, pollen FTLs are generated in the *quartet1* (*QRT1*) mutant background. Genetic disruption of the *QRT1* gene encoding a pectin methylesterase leads to physical attachment of the four products of male meiosis in pollen, enabling visualization of classical tetrad analysis of COs via two or three linked fluorescence T-DNA markers. Twelve and four tetrad classes are produced from three-color and two-color FTL intervals, respectively, according to CO position and number of COs within the interval. DeepTetrad, a deep learning-based image package, was developed to precisely analyze fluorescent pollen tetrad images of FTLs in a high-throughput manner (Lim et al., 2020). The pollen FTL system can also be adapted to detect single pollen fluorescence using flow cytometry in a wild-type background, facilitating high-throughput analysis of CO frequency (Yelina et al., 2013). Application of DeepTetrad or flow cytometry to the FTL system enables accurate measurements of CO frequency and interference in individual plants, suggesting that pollen FTL may be developed to screen CO frequency mutants in the future. The pollen FTL system was previously used to demonstrate a large increase in class II CO frequency when each or a combination of three anti-recombination pathways were genetically disrupted (Crismani et al., 2012; Girard et al., 2015; Séguéla-Arnaud et al., 2017). The pollen FTL system was also used to detect moderate changes in CO frequency at different temperatures (Lloyd et al., 2018; Modliszewski et al., 2018). Further, a pollen FTL (*CEN3*) spanning the centromere of chromosome 3 (YFP-Chr3:11115724, RFP-Chr:16520560) was used to reveal increased CO frequency around peri-centromeres in CHG methylation- or H3K9 dimethylation-defective mutants (Underwood et al., 2018). In addition, pollen or seed FTL systems can be combined with miRNA-induced gene silencing to examine the effects of meiosis-specific knockdown of essential or redundant genes on CO frequency along chromosomes (Kim et al., 2021; Nageswaran et al., 2021).

GENOME-WIDE MAPPING OF CROSSOVERS

In *Arabidopsis*, greater CO frequency occurs near both chromosome ends in male meiosis than in female meiosis (Giraut et al., 2011). The number of COs and their distribution along chromosomes in male and female meiosis were determined using BeadXpress technology with a genome-wide set of SNP markers (Drouaud et al., 2006; Giraut et al., 2011). Genomic SNP typing-based CO maps indicated that a large number of COs occurred in F_2 individuals and backcrossed plants of the *recq4 figl1* hybrid in *Arabidopsis*, and the *recq4* mutation led to a 3-fold increase in CO frequency in rice, peas, and tomato (Fernandes et al., 2018; Mieulet et al., 2018). In parallel, the genotyping-by-sequencing (GBS) method cost-effectively generated high-resolution genomic CO maps (Fig. 2E) (Rowan et al., 2015). The GBS method involves rapid extraction of F_2 individual genomic DNA, sequencing and indexed library construction, and running trained individual genome reconstruction (TIGER), a bioinformatics pipeline used to reconstruct mosaic genomes of F_2 individuals from sparse sequence coverage. Combining GBS with TIGER enabled a precise comparison of the positioning and number of COs in wild-type hybrids with those of diverse genetic backgrounds such as *zyp1, asy1, msh2, hcr1,* and *HEI10* mutants (Blackwell et al., 2020; Capilla-Pérez et al., 2021; Kim et al., 2021; Lambing et al., 2020; Nageswaran et al., 2021; Serra et al., 2018a; Zhu et al., 2021). GBS-based CO maps have provided the DNA sequence and chromatin features of CO hotspots in *Arabidopsis* (Shilo et al., 2015; Wijnker et al., 2013).

Recently, a linked sequencing technique was applied to a pool of pollen DNA from F_1 hybrids in *Arabidopsis*, generating a high-resolution CO landscape that was comparable to single pollen sequencing but more cost-effective (Luo et al., 2019; Sun et al., 2019). In addition, long-read sequencing technology has been adapted to directly sequence a pool of pollen DNA from F_1 hybrids to map genomic COs and altered structures (Naish et al., 2021; Wang et al., 2021). Genomic CO maps have led to the discovery of CO hotspots, including single CO hotspots that have been analyzed using the pollen typing method (Choi et al., 2013; 2016; 2017; Drouaud et al., 2013; Serra et al., 2018b; Yelina et al., 2012; 2015). At a single CO hotspot, DNA methylation or *arp6* mutation was shown to induce fewer COs (Choi et al., 2013; Yelina et al., 2015). As promoting COs in specific regions is of great interest in plant breeding, the CRISPR/dCas9 targeting approach was applied to increase CO frequency at a single CO hotspot in *Arabidopsis* using MTOPVIB (meiotic topoisomerase VIB)-dCas9 fusion, but the moderate effect reflected the complexity of meiotic recombination control (Yelina et al., 2021). Two MTOPVIB proteins interact with SPO11-1 and SPO11-2 topoisomerase-like proteins that catalyze meiotic DSB formation. The MTOPVIB-dCas9 was expressed during meiosis and targeted to *3a* CO hotspot via a guide RNA, which was expected to recruit SPO11-1 and SPO11-2, and subsequently induce DSBs and COs.

CONCLUSION AND FUTURE PERSPECTIVES

Recent advances in cytological, genetic, and genomic approaches for detecting COs have contributed significantly to our understanding of CO formation and meiosis in the model plant *Arabidopsis*. We here summarized the main methods of immnocytology, FTL systems and GBS methods for fast and accurate measurement of COs. The advanced approaches have allowed us to elucidate the roles of pro-CO, anti-CO and epigenetic factors in controlling of CO frequency and distribution. Along with super-resolution microscopy techniques, live imaging systems have been recently established to visualize the dynamics of chromosome behavior during meiosis (Prusicki et al., 2019). In the future, advanced genomic approaches, including meiosis-specific transcriptomics, single-cell RNA sequencing, and epigenome maps using INTACT (isolation of nuclei tagged in specific cell types), will provide important genomic information on CO formation that will help elucidate the molecular mechanisms controlling meiotic recombination in plants (Barra et al., 2021). As an efficient biochemical method for identifying new CO modulators, proximity labeling techniques can be adapted to generate protein interactomes for meiotic proteins in *Arabidopsis* (Mair and Bergmann, 2022). Moreover, genetic screening and identification of CO modifiers using FTLs and natural accessions will lead to the discovery of new pro-and anti-CO factors in *Arabidopsis*, further illuminating the CO pathways in plants. These noteworthy technical advances in detecting COs and isolating new CO modifiers will have profound implications for plant breeding and our understanding of meiotic recombination. Furthermore, manipulating the rate and positions of COs using CRISPR/Cas9-based genome editing methods will accelerate plant breeding and QTL (quantitative trait locus) mapping (Gao, 2021; Oh and Kim, 2021; Taagen et al., 2020).

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AUTHOR CONTRIBUTIONS

H.K. and K.C. wrote the manuscript. H.K. generated figures.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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