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REVIEW ARTICLE

Epigenetic regulation during meiosis and crossover

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Abstract
Meiosis is a distinctive type of cell division that reorganizes genetic material between generations. The initial stages of meiosis consist of several crucial steps which include double strand break, homologous chromosome pairing, break repair and crossover. Crossover frequency varies depending on the position on the chromosome, higher at euchromatin region and rare at heterochromatin, centromeres, telomeres and ribosomal DNA. Crossover positioning is dependent on various factors, especially epigenetic modifications. DNA methylation, histone post-translational modifications, histone variants and non-coding RNAs are most probably playing an important role in positioning of crossovers on a chromosomal level as well as hotspot level. DNA methylation negatively regulates crossover frequency and its effect is visible in centromeres, pericentromeres and heterochromatin regions. Pericentromeric chromatin and heterochromatin mark studies have been a centre of attraction in meiosis. Crossover hotspots are associated with euchromatin regions having specific chromatin modifications such as H3K4me3, H2A.Z. and H3 acetylation. This review will provide the current understanding of the epigenetic role in plants during meiotic recombination, chromosome synapsis, double strand break and hotspots with special attention to euchromatin and heterochromatin marks. Further, the role of epigenetic modifications in regulating meiosis and crossover in other organisms is also discussed.

Keywords Crossover · Recombination · Histone marks · DNA methylation · Meiosis · Epigenetics

Introduction

Meiotic division is essential in all the sexually propagating eukaryotes. Meiosis maintains stable chromosome numbers across generations, as it ensures that every gamete receives only half the number of chromosomes of a parent. Meiotic cells undergo single round of DNA replication and condensation before two rounds of chromosomal segregation (Yelina et al. 2015a, b; Dawe 1998; Mercier et al. 2015). The major differences between mitosis and meiosis are the ploidy of progeny cells and additional steps undergoing in Prophase I (Mercier et al. 2015). In *Arabidopsis thaliana*, meiosis starts with double strand breaks (DSBs) at the leptotene stage and recombination process commences. Pairing between homologous chromosomes occurs at zygotene via the polymerization of the synaptonemal complex (SC) and recombination proceeds. Synapsis completes by pachytene, SC disassembles in the diplotene stage and the chiasmata structure displays the crossover sites between homologous chromosomes. Chromosomal condensation occurs during diakinesis and bivalents are visible (Sims et al. 2021; Ross et al. 1996; Mercier et al. 2015). Segregation of homologs takes place during meiosis I and segregation of sister chromatids occur during meiosis II to generate 4 gametes. DSB repair happens through crossovers (COs) and non-crossovers (NCOs) (shown in Fig. 1). It's common in all the plants to have more DSBs than the resulting crossovers. Most of these breaks end up as Non-crossovers (NCOs) and sister chromatids are used as a template in this repair pathway. Not just crossing overs, non-crossovers (gene conversions) are also important for maintaining diversity (Mercier et al. 2015).

Meiotic crossovers use homologous chromosomes as a template and shuffle the genetic information. It is a necessary for each pair of homologs to undergo a minimum of one crossover for proper segregation i.e., obligatory crossing over (Darlington et al. 1932). Recombination has been

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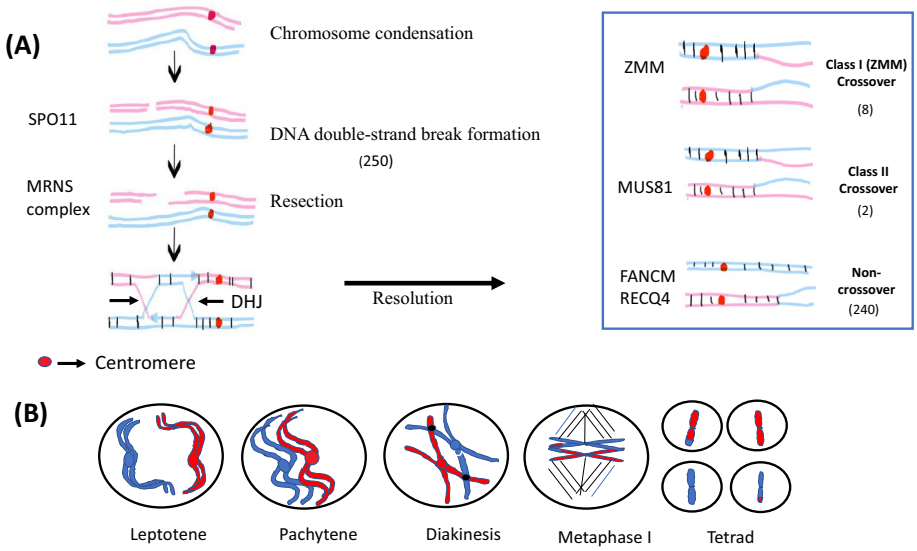

Fig. 1 Steps occurring in the Arabidopsis recombination process is shown in brief. **A** Homologous chromosomes are shown in two contrasting colours (blue and pink). Estimated frequency of each crossover pathway is represented in brackets. The prominent proteins involved in each step are mentioned on the left side. (MRNS complex- MRE11-RAD50-NBS1/XRS2-SAE2/COM1, MUS81-MMS AND UV SENSITIVE81, FANCM-FANCONO ANEMIA COMPLEMENTATION GROUP M, DHJ-Double Holliday Junction). **B** Some of the important meiotic stages are represented at the lower panel of the figure

one of the major factors in driving speciation and variation. However, its effect on the genome varies as the recombination sites are unevenly distributed. Certain regions of the genome are more prone to crossover events than others and these regions are known as recombination hotspots (Marand et al 2019). In most flowering plants, crossing overs are abundant in sub-telomeric and chromosomal arms whereas crossovers are scarce in ribosomal DNA, pericentromeric and centromeric regions (i.e., U-shaped trend). The exceptions are plants like *Nelumbo nucifera* and *Camellia sinensis* which have higher recombination rates in the middle of the chromosome (Brazier and Glemin et al. 2022). The longer the chromosomal length, the higher the chance of distal bias (Haenel et al. 2018).

Epigenetic modifications are known to have been involved in meiotic regulation and crossover (Yelina et al. 2015a, b). It refers to a layer of information that exists beyond what is encoded in the DNA sequence. Epigenetic modifications can be divided into four categories: DNA methylation, histone post-translational modifications, chromatin remodelling and non-coding RNAs (ncRNAs) (Portela and Esteller 2010; Dziegielewski and Ziolkowski 2021). All these modifications are very dynamic in nature which means they could be stage-specific, tissue-specific, loci-specific. These dynamic structural changes can affect processes like transcription, DNA repair, chromosome segregation and transposon movements. Chromatin states in a chromosome are differentiated into euchromatin (enriched with active genes), facultative heterochromatin (enriched with repressed genes) and constitutive heterochromatin (enriched with transposons and repeats). Epigenetic modifications regulate directly (in cis) or indirectly through regulating meiotic gene expression (in trans) (Mirouze et al. 2012). Most of the studies on plant meiosis are available in male meiosis. It is technically difficult to isolate intact embryo sac mother cell with analysable chromosomes (Koul et al. 2023). The recombination steps take most of the time of meiotic phase indicating that it's a time taking and highly regulated process (Prusicki et al. 2019; Osman et al. 2021).

All the epigenetic classes interplay with each other and reinforce through many positive and negative feedback mechanisms (Portela and Esteller 2010). The epigenetic factors initiating and determining the crossover sites in flowering plants still remain a puzzle.

Meiosis in plants: an overview

In plants, meiotic studies with recombination patterns are available in Arabidopsis, barley, wheat, maize, rice, potato and tomato (Anderson et al. 2014; other references are mentioned in Table 1). Meiotic events and genetic regulation of meiotic division in plants are more or less the same as observed in Arabidopsis. The meiotic processes of Arabidopsis are briefly discussed here. *Arabidopsis thaliana* stands out as a model plant for studying chromatin anomalies and meiotic recombination because of the already available resources with chromosome spreading protocols (Ross et.al. 1996), crossover numbers (Francis et al. 2007) and lesser chromosomal number (2n = 10). The process of meiotic recombination involves the exchange of genetic material between homologous chromosomes that occurs in several steps, which can be summarized as follows: (1) Chromosome condensation (2) DNA double-strand break formation (3) Resection (4) Strand invasion and double holiday junction formation (5) Resolution (Fig. 1).

During early prophase I of meiosis, the replicated homologous chromosomes condense. Double strand breaks (DSBs)

Table 1 Factors influencing crossover positions like DNA sequence motifs, histone modifications and transposons that are correlated with recombination hotspots are specified in the table

Plant name	Sequence motifs	Modifications positively correlated	Modifications negatively correlated	Transposons	References
Arabidopsis (*Arabidopsis thaliana)*	A-rich, CCN and CTT repeat	H3K4me3, H2A.Z,	H2A.W6, H2AW7, H3K9me2	Helitron/Pogo/Tc1/Mariner DNA transposons	Naish et al. (2021), Shilo et al. (2015)
Wheat *(Triticum aestivum)*	A-rich, CCG repeat,	H3K27me3	H3K27me2/1; H3K9me2	Mariner TE	Liu et al. (2021), Darrier et al. (2017), Tock et al. (2021)
Maize (*Zea mays*)	GC rich (MHS)	H3K4me3 (~20%)		Gypsy	He et al. (2017)
Rice (*Oryza sativa*)	GGC, TACT	H3K4me3, H3K27me3, H3K9ac, H4K12ac,	H3K36me3	Stowaway and PIF/Harbinger	Si et al. (2015), Marand et al. (2019)
Potato (*Solanum tuberosum)*	poly-AG, poly-A/T, CCN repeat	H3K4me3	–	Stowaway	Marand et al. (2017)
Soybean (*Glycine max)*	poly-A and AT-rich motifs	H2A.Z, H3K4me3, H3K9me3, H3K27me3, H3K14ac, H3K27ac, H3K56ac H4K12ac	H3K36me3 and H3K4me1	–	Ma et al. (2023)
Tomato (*S. lycopersicum)*	CCN repeats, poly-A/T and AT-rich motifs	–	–	–	Demirci et al. (2017)

– indicates data is not available

are the starting point for meiotic recombination. They are catalysed by SPORULATION 11 (SPO11) (Grelon et al. 2001). DSBs are then processed by MRE11-RAD50-NBS1/XRS2-SAE2/COM1 (MRNS) complex and EXO I generates 3' overhang single-stranded DNA (ssDNA). The 3' overhangs are bounded by heterotrimeric replication protein A (RPA) complex and loading of recombinases like RADIATION SENSITIVE51 (RAD51) and DISRUPTED MEIOTIC cDNA1 (DMC1) takes place during leptotene stage (Li et al. 2004; Couteau et al. 1999). In pachytene, the synaptonemal complex (SC) is completely formed between aligned maternal and paternal chromosomes forming synapsis between the bivalents. SC is the zipper like tripartite proteinaceous structure formed between chromatin loops made up of central elements and lateral axes. One of the single-stranded DNA ends invades the homologous chromosome and pairs up with a complementary strand on the homologous chromosome. DNA synthesis follows, extending the invading strand and displacing the original strand of the homologous chromosome. This leads to the creation of the Holliday junction, a structure where the two DNA duplexes are intertwined. In diplotene, the Holliday junction is resolved in two ways: either by cutting the junction horizontally to produce two non-crossover products or by cutting it vertically to produce two crossover products. As the homologous chromosomes separate, X-shaped structures called chiasmata are formed. Chromosomes further separate and condense by diakinesis stage. The resulting chromosomes are different from the original chromosomes as they contain a mixture of genetic information from each parent (for a detailed recombination process, see the review; Mercier et al. 2015).

Homologous recombination (HR) happens in both somatic cells and meiotic cells. In somatic cells, it is predominantly repaired through Non-Homologous End Joining pathway (NHEJ) and rarely through HR. Sister chromatids act as a template in somatic cells, while homologous chromosome act as a template in meiotic cells. It has been known that DMC1 favours inter-homolog recombination (IH) and crossing over in meiotic cells while RAD51 favours sister chromatid recombination in both mitotic and meiotic cells. RAD51 and DMC1 function independently and are spatially separated during meiosis (Kurzbauer et al. 2012). Their foci do not colocalize and the DMC1 triggers down-regulation of RAD51 exchange activity through an unknown signalling pathway however the presence of RAD51 is essential during chromosomal strand exchange (Da Ines et al. 2022).

In Arabidopsis, on an average of 250 DSBs, 12 recombination events takes place per meiosis. Out of these 12, 10 are class I COs and the other two are class II COs (Mercier et al. 2015). Homolog-dependent repair of a DSB can occur through Class I, Class II and NCO pathways. Class I is interference

dependent and relies on a group of proteins called ZMM and two other conserved proteins called MutL HOMOLOGS (MLH1 and MLH3) (Jean et al. 1999; Mercier et al. 2015). That is why class I is also called as ZMM pathway. Class II is interference independent (little or no interference) and MMS AND UV SENSITIVE81 (MUS81) contribute to it (Higgins et al. 2008; Mercier et al. 2015). Sturtevant (1915) described interference as follows: "The occurrence of one crossing-over in a given chromosome pair prevents another nearby" . Class I contributes to above 70–80 per cent of all the crossovers and class II plays just a minor role. Although Class II is interference independent, class I and class II COs can detect and interfere with each other (Anderson et al. 2014). FANCONO ANEMIA COMPLEMENTATION GROUP M (FANCM) and RECQ4 helicases direct recombination intermediates from class II CO pathway to synthesis-dependent-strand-annealing NCO pathway (Crismani et al. 2012; Lorenz et al. 2012; Mercier et al. 2015). Apart from these three, there is a non-interference pathway described in *Solanum lycopersicum* called FANCD2 (FANCONO ANEMIA D2) (Kurzbauer et al. 2018). There is limited information available about this pathway. It is unclear why many pathways have to be evolved and interplay with each other. According to population genetics perspective, the substrate of each pathway could have the deciding factor in evolving different pathways (Ziolkowski et al. 2023). Ribosomal DNA (rDNA) arrays regulate their crossover numbers through NHEJ pathway (Sims et al. 2019).

In Arabidopsis, crossovers can be estimated through classical genetic analysis using segregation of markers in the progeny, chiasmata counting, visual assay of fluorescent pollen tetrads (FTLs) and immunostaining of MLH1 foci (Berchowitz and Copenhaver 2008; De Muyt et al. 2009; Mercier et al. 2015; Francis et al. 2007). FTLs are as best as *Chlamydomonas reinhardtii* zoospores and *Saccharomyces* tetrads systems to determine the crossover rates from a region of interests (Francis et al. 2007). Deep tetrad software ease the process through it's high-throughput analysis of tetrads and yield very quick results (Lim et al. 2020). Flow cytometry based protocols can achieve high-throughput analysis but they miss out on calculating double crossovers and gene conversion measurements (as pollen tetrads -*qrt1* mutants are not used in the protocol) (Yelina et al. 2013). Fluorescent antibodies usage against MLH1 and MUS81 foci gives the number of class I COs and class II COs, respectively (Mercier et al. 2015).

Factors affecting crossover positioning

Epigenetic modifications, such as histone modifications, DNA methylation and DNA remodelers can influence crossover positioning by altering the accessibility of DNA to the recombination machinery. The process of homologous chromosome pairing during meiosis can influence the positioning of crossovers. Telomeres attach to the nuclear membrane in the early prophase I stage and form bouquet-shaped chromatin organisation. In grasses, there is asynchronous timing of axis maturation and SC formation leading to early maturation and localisation of ZMM proteins in distal regions. It is believed early interaction can lead to early designation of crossover sites (Higgins et al. 2012, 2022). Pro-Crossover ZMM class of proteins like HUMAN ENHANCER OF INVASION 10 (HEI10) diffuse and coarsen across the chromosome. Only these foci turn into crossovers (Morgan et al. 2021). Crossing over in plants is tightly regulated by the ZMM pathway and synaptonemal complex (Durand et al. 2022). The DNA sequences called DNA motifs or sequence motifs, are found in the vicinity of crossover hotspots. They vary from plant to plant. Surprisingly, transposons also overlap with crossover locations. (shown in Table 1). Structural variants like inversions, transpositions and indels can also show local CO suppression and determine CO positioning (Rowan et al. 2019).

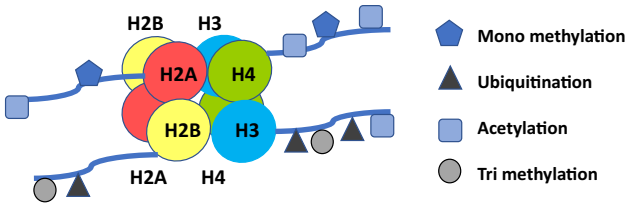

Fig. 2 Diagram depicting histone octamer and displaying various histone modifications in their tails. The histone octamer shown in the figure is a 3-D version of it (H1 linker is missing). Various covalent modifications are represented in different shapes and shades

Epigenetic factors involved in the regulation of meiosis

Histone modifications in meiotic regulation

In eukaryotes, DNA wraps around the positively charged proteins called histones and this DNA–protein complex is called chromatin. About 146 bp of DNA make a tight 1.65 negative super helical turns around an octamer of core histone proteins. The histone octamer consists of a central heterotetramer of histones H3 and H4, flanked by two heterodimers of histones H2A and H2B (i.e., two copies of H3, H4, H2A and H2B each) (Fischle et al. 2003).

A variety of posttranslational modifications have been discovered on tails such as acetylation, methylation, ubiquitination, phosphorylation, sumoylation and ribosylation (some of the marks are shown in Fig. 2). The histone modifications are seen in both amino-terminal (N-terminal) and carboxy-terminal tails. Modifications are abundant in the H3 histone N-terminal tail. The modifications acting as hallmark

of actively transcribed regions are called active chromatin marks and the marks abundant in non-transcribed regions are called repressive chromatin marks. These modifications are written by writer proteins, read by reader proteins and removed by eraser proteins. Some of the proteins can do multiple roles. All these effector proteins help to precisely balance the location of histone marks. For example, SET DOMAIN GROUP8 (SDG8) in Arabidopsis encodes for a histone methyl transferase that have both a writer domain called SET domain and a reader domain called CW domain. JUMONJI 30 (JMJ30), a histone demethylase acts as an eraser for the same histone mark H3K36me3 (Cheng et al. 2020).

Acetylation of histone proteins involves the addition of an acetyl group to lysine residues, which neutralizes the positive charge of the lysine (+1 to 0) and weakens the interaction between the histone and the negatively charged DNA. This leads to a more relaxed chromatin structure and increased accessibility of the DNA to transcription factors and other regulatory proteins. Methylation of histone proteins occurs on lysine or arginine residues and can have different effects depending on the specific site and degree of methylation. Phosphorylation of histone proteins occurs on serine, threonine, and tyrosine residues and can affect chromatin structure and gene expression in a variety of ways (Peterson et al. 2004). The role of histone marks can change from one organism to the another. H3K4me2 is an active chromatin mark in animals and Arabidopsis but it acts as a repressive mark in rice (Liu et al. 2019; Cheng et al. 2020; Tock et al. 2021). Histone euchromatin (active) and heterochromatin (repressive) marks are represented in Table 2.

Table 2 Classification of the major histone marks in Arabidopsis based on their transcription function (Cheng et al. 2020; Tock et al. 2021; Zhang et al. 2023; Liu et al. 2016)

Euchromatin marks	Heterochromatin marks
H2Bub1	H3K9me1/2
H3K4me1/2/3	H3K27me1/2/3
H3K36me2/3/ac	H2Aub1
H3 K9/K14/K18/K23/K27ac H4 K5/K8/K12/K16/K20ac	

Cohesin subunit AtSYN4 recruits Histone H2A monoubiquitination (H2Aub1) mark specifically into the genomic loci showing that there could be prominent interplay between cohesin subunits and histone modification enzymes (Zhang et al. 2023). Transcription factors also recruit the chromatin modifying enzymes leading to the accumulation of specific histone marks (Zhang et al. 2015) (Both cohesin and transcription factor recruitment are shown in Fig. 3).

In Arabidopsis, recombination hotspots tend to be located in gene-rich regions of the genome and these are highly correlated with active chromatin modifications, including H2A.Z, histone H3 Lys4 trimethylation (H3K4me3) (Choi et al. 2013), low nucleosomal density, low DNA methylation (Choi et al. 2018; Underwood et al. 2018).

In Arabidopsis, Histone H3 Lys 4 trimethylation (H3K4me3), an active euchromatin mark, is highly enriched near SPO11-1 oligos but not highly correlated with double strand breaks at fine level. In the DSB site, exonucleases release SPO11-oligonucleotide complexes. These oligos provide a high-resolution profile of meiotic DSB patterns genome-wide (Choi et al. 2018). SET DOMAIN GROUP 2 (SDG2) encodes for the H3K4me3 mark and the loss of function mutant *sdg2* has not shown any defect in meiosis. Its role is limited to post meiotic microspore development, especially chromatin decondensation in the pollen vegetative nucleus (Pinon et al. 2017). In *Saccharomyces cerevisiae* (budding yeast), H3K4me3 is enriched in the vicinity

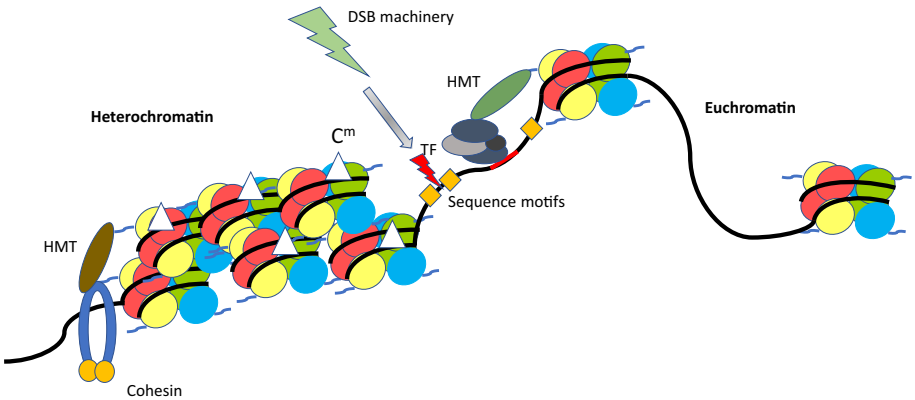

Fig. 3 Diagram showing the DSBs generally localise with euchromatin features like low nucleosomal occupancy, DNA methylation (C^m) and sequence motifs. Most of the DSBs in Arabidopsis overlap with promoter regions. Cohesins and Transcriptions factors (TF) recruit various kinds of histone modification enzymes. Histone methyl transferases-(HMTs) responsible for euchromatin is shown in green colour and for heterochromatin is shown in brown colour

of DSB sites and the mutant showed a drastic reduction in DSBs (Borde et al. 2009). Spp1 subunit of COMPASS complex recognises H3K4me3 rich hotspots and Mer2 axis and recruits the DSB site to the chromosomal axis for the action of Spo11 (Acquaviva et al. 2013; Lambing et al. 2020; Yelina et al. 2015a, b). Surprisingly in *Schizosaccharomyces pombe* (fission yeast) H3K9ac is more significantly enriched than H3K4me3 in hotspots (Yamada et al. 2013). PR DOMAIN CONTAINING 9 (PRDM9) dependent H3K4me3 majorly determines recombination hotspots in humans and mice (Baudat et al. 2009). PRDM9 (meiosis-specific histone lysine methyltransferase with SET domain) binds to DNA at the hotspot and trimethylates H3K4 as well as H3K36, which causes nucleosomes to move apart creating favourable site for SPO11 to produce double strand break (Parvanov et al. 2010; Diagourage et al. 2018; Bhattacharya et al. 2019; Powers et al. 2016) (Fig. 4). DSBs are formed in the *prdm9* mutant, but are formed at different locations. It means PRDM9 does not initiate the meiotic recombination but is required only for determination of hotspot position and DSBs (Brick et al. 2012). CXXC finger-domain protein, a component of H3K4 methyltransferase in mice is involved in regulating meiotic gene expression (Ki et al. 2022). However, plants lack PRDM9 homologs and CXXC kind of finger-domain proteins. Almost in all the organisms H3K4me3 is seen in the vicinity of recombination hotspots but the mark is probably controlled through different molecular mechanisms.

In Arabidopsis, Histone 3 lysine 9 dimethylation (H3K9me2), a repressive mark, is abundant in transposable elements and repetitive sequences. H3K9me2 mark is controlled by methyl transferases called SUPPRESSOR OF VARIEGATION HOMOLOG 4/KRYPTONITE (KYP/SUVH4/5/6) and histone demethylases called IBM1 (INCREASE IN BONSAI METHYLATION). Mutants of

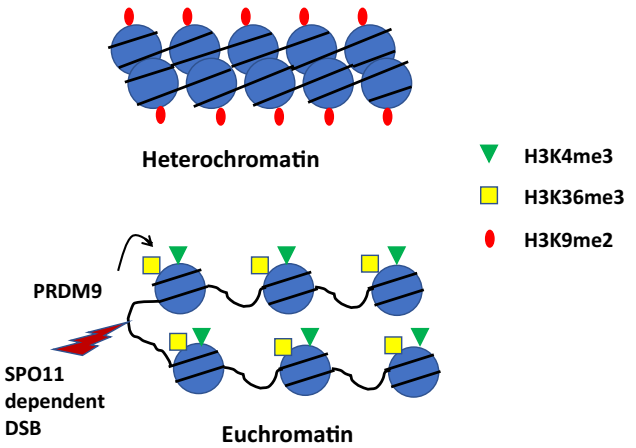

Fig. 4 Model showing the role of histone marks and PRDM9 in SPO11-dependent double strand break in mammals

KYP/SUVH4/5/6 have shown an increase in recombination without any chromosomal defects (Underwood et al. 2018) whereas demethylase mutant *ibm1* has displayed incomplete synapsis, chromosome entanglement, reduction of recombination during meiosis and fertility. The number of chiasmata per cell in *ibm* mutants is less compared to the WT (He et al. 2022). These defects are due to IBM1-mediated alterations in gene expression and interaction with cohesin cofactor PDS5s in downstream (Cheng et al. 2022; He et al. 2022). It means that timely maintenance of H3K9 demethylation is more important than H3K9 methylation in plant meiosis. Whereas in mammalian meiosis H3K9 methylation is important for prophase progression. Loss of heterochromatic H3K9 methylation leads to improper synapsis, leading to abnormal chromosome segregation and infertility in mice (Tachibana et al. 2007; Takada et al. 2021). Similar to Arabidopsis, drosophila and fission yeast H3K9me2 mutants are reported to have more crossovers (Ellermeier et al. 2010; Peng and Karpen 2009). In fission yeast, H3K9 methylation marks attract mitotic cohesion complex and limit DSBs around centromeres. It's unknown in plants how H3K9me2 is limiting DSBs (Nambiar and Smith 2018). In higher organisms, pericentromeres are evolved to have 100 times lower crossovers than chromosomal arms. Recombination in pericentromeres is known to have mis-segregation and aneuploidy (for example: Down syndrome) (Nambiar and Smith 2016). Whereas in Arabidopsis, cytologically there are no chromosomal defects found despite increasing pericentromeric crossovers (Underwood et al. 2018).

REC8, a sister chromatid cohesin is associated with suppression of DSBs and meiotic crossovers (Lambing et al. 2020). In fission yeast, REC8 is enriched in the centromeres. In H3K9me2 mutants of fission yeast, REC8 loading is greatly reduced and leading to chromosomal segregation defects (Ellermeier et al. 2010). Despite the fact that the REC8 and H3K9me2 peaks overlap in Arabidopsis, REC8 loading remained normal in H3K9me2 and non-CG DNA methylation mutants. It's also possible that other heterochromatin marks are driving the REC8 localisation in Arabidopsis (Lambing et al. 2020). In yeast, H3 histone modifications are involved in meiotic recombination and highly acetylated in recombination hotspots (Borde et al. 2009; Merker et al. 2008; Yamada et al. 2004). However, in Arabidopsis, H3 Histone hyperacetylation with overexpression line *gcn5-related histone N-acetyltransferase (mcc1),* displayed difficulty in segregation, altered chiasmata distribution, leading to the abortion of half of the gametes (Perrella et al. 2010).

In maize, DSBs are distributed uniformly in a chromosome but crossovers follow the usual U-shaped trend (higher CO rate in chromosomal ends and lower CO rate in centromeres). Not even 20 percent of hotspots are associated with H3K4me3 mark. As the majority of Maize genome is filled with transposons, DSBs are abundant in transposons

but only the genic DSBs turned into crossovers (He et al. 2017). So, with all these unique features maize has to be considered as an outlier. In allopolyploids like wheat, spatiotemporal asymmetry of meiotic programs is possibly favouring distal pattern of crossover localisation (Higgins et al. 2022; Osman et al. 2021). Along with the distinct pattern, H3K4me3, H3K9me3, H3K27me3 and H3K27me2 marks are also preferentially enriched in distal parts of wheat chromosomes (Osman et al. 2021; Tock et al. 2021). Facultative heterochromatin mark H3K27me3 is positively correlated along with (ASYNAPTIC 1) ASY1 and DMC1 peaks with crossover regions. This polycomb repressive mark is enriched in the crossover active distal regions of chromosomes and is highly associated with disease responsive genes (nucleotide binding leucine rich-repeat proteins) (Tock et al. 2021; Liu et al. 2021). H3K27me2 is also enriched in the distal area of chromosomes, but it's associated with crossover suppression. Both the marks are mutually exclusive and the H3K27me2 role in supressing crossovers is also observed in Arabidopsis and maize (Liu et al. 2021).

In rice and soybean, Histone 3 lysine 36 trimethylation (H3K36me3), an active transcription mark is negatively correlated with crossovers (Ma et al. 2023; Marand et al. 2017). Even in yeast, H3K36me3 negatively correlates with DSBs and meiotic crossovers (Hansen et al. 2011; Merker et al. 2008). Further, H3K36me3 mark decreases resection and promoting non-homologous end joining pathway (NHEJ) (Pai et al. 2014). The immunolocalization analysis of various H3 methylation and acetylation modifications throughout all phases of meiosis in *Aegilops sp*., *Secale cereale* and *Arabidopsis thaliana* showed that their distribution and dynamics of histone marks are species specific and stage specific. This implies that there is an evolutionary divergence in histone language (Oiliver et al. 2013).

DNA methylation in meiotic regulation

DNA methylation mainly occurs at the fifth position of cytosine, resulting in 5′-methylcytosine (5-mC). In general, hypermethylation and hypomethylation are found in heterochromatin and euchromatin regions, respectively (Zhang et al. 2018). In plants, DNA methylation occurs at cytosine residue in all three sequence contexts such as symmetric CG, CHG and asymmetric CHH (where H is any nucleotide except G) while CG methylation is predominant in animals. As of now, only 5 methyltransferases are known in Arabidopsis. They are METHYLTRANSFERASE 1 (MET1), CHROMOMETHYLASE 2 (CMT2), CHROMOMETHYLASE 3 (CMT3), DOMAINS REARRANGED METHYLTRANSFERASE 1 (DRM1) and DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2). DRM2 is responsible for DNA methylation in all three sequence contexts (CG, CHG and CHH), whereas MET1 and CMT3 maintain CG and CHG methylation, respectively. CMT2 and DRM2 are jointly responsible for maintaining CHH methylation in long heterochromatic transposable elements (TEs) and short euchromatic TEs, respectively (Fang et al. 2021).

In Arabidopsis, the centromeres are highly heterochromatic regions that are epigenetically silenced by H3K9me2 and DNA methylation in CG and non-CG sequence contexts. MET1 maintains CG methylation all over the chromosome (Mirouze et al. 2012). Loss of CG methylation in *met1* is shown to have more SPO-11-1-oligos, reduction of nucleosome density, slight gain of H3K4me3 and transcriptional reactivation of repetitive sequences in centromeres. The *met1* mutant got triggered into crossover remodelling and resulted in having complex phenotype of increased chromosomal arms, centromere recombination and decreased pericentromeric recombination (Choi et al. 2018; Yelina et al. 2012). In total, DSB foci and crossover number remain the same but the crossovers are redistributed to chromosomal arms from pericentromeres. The redistribution is limited to only interfering crossovers (class I COs) (Choi et al. 2018; Yelina et al. 2015a, b). In somatic cells of *met1* mutant, there is a redistribution of epigenetic marks among H3K9me2, DNA methylation and H3K27me3 in PcG target genes (Deleris et al. 2012). Ectopic H3K9me2 marks noticed in the proximal region of chromosomes could be due to the redistribution of chromatin marks. Along with the alteration in chromatin modifications, crossover interference mechanisms might be responsible for this complex phenotype (Yelina et al. 2015a, b).

The chromatin remodeler DDM1 (DECREASED DNA METHYLATION 1) gene is responsible for DNA methylation and heterochromatin maintenance. In the *ddm1* mutant, CG methylation is reduced throughout the genome especially in heterochromatin regions. FTL markers between euchromatin regions showed more recombination rate but the pericentric heterochromatin FTL markers showed similar recombination rate as of control (Bessudo and Levy 2012). DDM1 is also responsible for maintaining classical Mendelian trait segregation. This is possibly through excessive gene conversion happening in the heterochromatin (Ali et al. 2021). A similar study in mouse, DNA methylation mutant *dnmt3*, has shown increased DSBs formation and gain of H3K4me3 marks in retrotransposon regions (Zamudio et al. 2015).

The mutation in the H3K9 methyltransferase (KYP/SUVH4/5/6) or the CHG methylation maintenance gene CMT3 (CG methylation remains same), showed a significant increase SPO11-1-oligos and crossovers in pericentromeric regions. Both class I and class II crossovers are elevated in the mutant (Underwood et al. 2018). Overall, both CG and non-CG methylation are able to inhibit DSBs, but only CHG methylation has the ability to inhibit crossovers at pericentromeric region (Choi et al. 2018). In somatic cells, CHG

methylation and H3K9me2 are mutually linked and dependent on each other. Proteins encoding these epigenetic modifications are able to recognise each other (Du et al. 2012). Additional binding proteins like AGENET DOMAIN CONTAINING P1 (AGDP1) also link H3K9me2 and non-CG DNA methylation (Zhang et al. 2018). A positive reinforcing loop is noticed in meiotic chromosomes also i.e., promoting each other's presence (Underwood et al. 2018). In all the plants studied, methylation has been negatively impacting crossing over. An unusual occurrence in the form of CHH and CHG methylation peaks is observed in rice hotspot regions (Marand et al. 2019). Rate of recombination in epigenetic mutants of Arabidopsis, categorized by region of chromosome, are represented in Table 3.

Histone variants in meiotic regulation

In addition to histone covalent modifications, there is an additional tier of variation called histone variants. Histone variants are versions of proteins that differ from the canonical histones in their amino acid sequence. Dozens of histone variants like H2A, H2B and H3 families have been discovered in eukaryotes (for example, H2A.Z) (Jamge et al. 2023). There is a great correlation and interplay between Histone modifications and these variants, which means that a variant is strongly associated with a specific mark (Loppin et al. 2020). For example, H3.3 is strongly associated with H3 acetylation and H3K36 methylation, whereas H3.1 is associated with H3K27me1. Histone variants can be incorporated into chromatin in a replication-independent manner, meaning that they are added to pre-existing chromatin rather than during DNA replication. This allows cells to modify chromatin structure in response to different signals and cellular states (Jamge et al. 2023).

Histone variant H2A.Z foci colocalize with crossover regions, DMC1 and RAD51. The knockdown mutant (*arp6*) has shown low DSB which led to low crossover numbers (Choi et al. 2013). ACTIN RELATED PROTEIN 6 (ARP6), a subunit of SWR1 ATP dependent remodelling complex is necessary for H2A.Z deposition and regulates meiotic gene expression during megasporogenesis (Qin et al. 2014). In fission yeast, the variant promotes chromatin compaction through cohesins (Yamada et al. 2018). Although the variant promotes DSBs, it is not enriched around the hotspots (Yamada et al. 2018). Phosphorylated H2A.X signals DNA damage response and invites chromatin remodelling complexes (Rogakou et al. 1998; Kuo 2021). This mark is generally used in immunolocalization studies to determine DSBs (Higgins et al. 2012). H2A.W, a heterochromatin mark is independent of other heterochromatin features and causes heterochromatin condensation (Yelagandula et al. 2014). *HTA6 and HTA7* are the two major genes responsible for H2A.W variant deposition and proteins are enriched on the pericentric heterochromatin (Yelagandula et al. 2014; Kuo 2021). The recombination rate is increased only in the heterochromatin regions of *hta6-1 hta7* mutants although the mutants showed normal cytology. Double mutants of the *hta7 cmt3* did not show to any significant rise in crossovers compared to the single mutant (*hta7*). The overall study has shown that the H2A.W variant is a repressor of meiotic recombination (Kuo 2021).

Noncoding RNAs in meiotic regulation

Noncoding RNAs are classified as small RNAs (sRNAs), medium size ncRNAs (mncRNAs) and long-size ncRNAs (lncRNAs). A large number of non-coding RNAs are upregulated in the events of meiosis. They have a prominent role in controlling meiotic gene expression, chromosome condensation and centromere organisation. Transcriptome studies in various plants have shown the significance of noncoding RNAs in meiosis (Jiang et al. 2023; Dziegielewski and Ziolkowski 2021). Small interfering RNAs (siRNAs) are important for transposon silencing and DNA methylation. Mutants of miRNA biogenesis and function, *dcl*, *hyl1*, *hen1*, *hst* and *ago1,* have shown higher expression of *SPO11*, *DMC1*, *RAD51*, *MSH4*, and *MUS81* (Dziegielewski and Ziolkowski 2021).

Other factors affecting crossover rate

Chromatin loops

Meiotic chromosomes are organized in loops and attached to the chromosome axis which interacts with chromatin to regulate meiotic recombination. At the macro level,

Table 3 Recombination rate in various Arabidopsis epigenetic mutants is summarised in accordance with the chromosomal region. Distal region includes chromosomal arms and subtelomeres

Mutants	Pericentromeres	Distal region	References
ddm1	Same	Rise	Bessudo and Levy (2012)
met1 (CG methylation)	Fall*	Rise	Yelina et al. (2012), Yelina et al. (2015a, b)
cmt3 (non-CG methylation)	Rise	Fall	Underwood et al. (2018)
kyp/suvh4/5/6 (H3K9me2)	Rise		Underwood et al. (2018)

*indicates CO rate is increased in centromeric region of the mutant

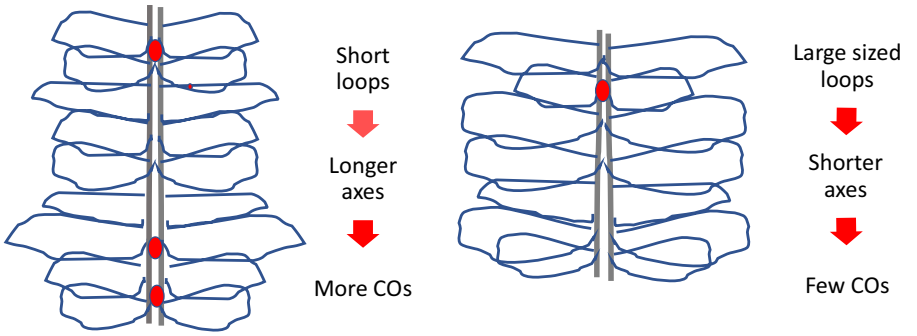

Fig. 5 A model explaining relationship between chromatin loops and crossover number: length of loops determines the axes length thereafter crossover number. CO sites are represented with red dots on axes

chromosomes appear as X-shaped structures. But the chromatin is packed into various kinds of loops to serve different functions. Larger loops form shorter axes, thus leading to fewer COs and vice-versa (Wang et al. 2019) (Fig. 5). Anchor regions of long-range cis chromatin loops are associated with H3K27me3 and maintain chromatin organisation in Arabidopsis (Sun et al. 2023). H3K27me3 deposition also influences chromatin compartmentalisation and gene co-regulation (Huang et al. 2021). There is a need to verify the role of the dynamic chromatin loops in choosing and initiating crossovers.

Abiotic stress

Meiotic crossover frequency is plastic and modulated by different factors like age, temperature, nutrient availability, developmental stage, and behaviour (Modliszewski and Copenhaver 2017; see the review). Crossover rate in Arabidopsis male meiosis follows a U-shaped trend in response to temperature range between 5 and 28 °C. CO rate is highest at 5 °C and 28 °C but the lowest at its optimal growth temperature 20 °C (Lloyd et al. 2018). The observed high meiotic crossover frequency at elevated temperature (28 °C) is derived from only the type I pathway. There is no increase in ds breaks, just the non-crossovers are converted into crossovers in this scenario (Modliszewski et al. 2018). Heat stress can also induce altered transposon activation, leading to the rearrangement of chromatin organisation (Sun et al. 2020). This can lead to chromatin decondensation and the activation of heterochromatin transcription (Tittel-Elmer et al. 2010).

Sudden exposure to temperature stress severely decreases the duration of early prophase stages, but the crossover maturation phases got prolonged. Mutants related to the recombination pathway like *spo11-1, dmc1, msh4* did not show this pattern, implying that prolong maturation phase is recombination-dependent (Braet et al. 2022). Heavy stress inhibits meiotic recombination via reduced ds breaks and homolog asynapsis. In barley, the distribution of recombination events is shifted to centromere under high temperature (Philips et al. 2015). The same shifting pattern is noticed in wheat under temperature stress, but it's not as significant as in the case of Barley (Coulton et al. 2020). In crop plants, many of the essential genes lie in recombination cold spots. Heat treatment can give different combinations of progeny due to the change in crossover distribution. Although the mechanisms are unknown, the mild heat stress can be tested in heterosis programmes.

Conclusion and future perspective

Meiotic recombination in plants is tightly regulated at different levels. It's maintained by various genetic, epigenetic, genome structure and environmental factors. Epigenetic factors influence the recombination landscape directly as well as indirectly through meiotic gene expression (overall model is shown in Fig. 6). On a local scale, crossover positioning is majorly done by histone marks, DNA methylation and structural variants. On a broad scale, the spatiotemporal asymmetry model and diffusion mediated ZMM proteins coarsening model are perfectly explaining the distal region preference in crossovers and crossover interference. Only grasses have shown additional factors like time of meiotic events, pre-meiotic DNA replication along with usual chromatin features and pro CO genes (Higgins et al. 2022). Pro crossover enzymes like HEI10 and anti-crossover factors like RECQ4 control the crossover positioning at the genome

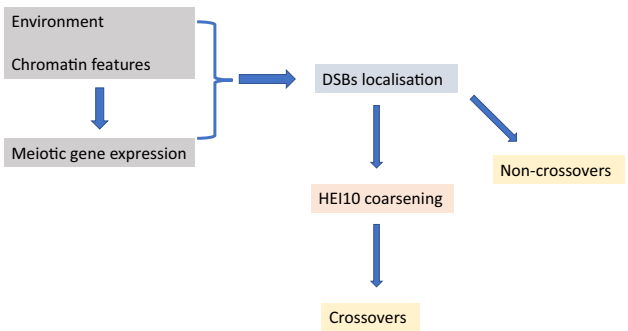

Fig. 6 Overall model showing the crossover regulation in Arabidopsis

level (Morgan et al. 2021). Positioning is initially determined by DSB sites and pro-crossover enzymes like HEI10 in a suitable chromatin environment do the next layer of selection. The accumulation of HEI10 determines the location of crossovers. The factors determining the location of HEI10 accumulation and coarsening are unknown.

Chromatin features can influence DSB proteins, recombination machinery and meiotic genes. The number of DSBs influences the total number of crossovers (Xue et al. 2018). Through this web of interactions, chromatin features control crossing over in one way or the other. Deciphering the chromatin features and mechanisms involved in crossover mechanisms will ease breeding programmes and help us to make smart crops. Increasing crossovers in pericentromeric regions has been achieved through various heterochromatin mutants in Arabidopsis. The heterochromatin marks (like H3K9me2) are also responsible for heterochromatin stability, so transferring these results into food crops has to be done in a careful manner (Peng and Karpen 2009). There is also a great variation in epigenetic control of meiosis among the model organisms. Only yeast has shown great similarity with recombination control as in plant systems. Undoubtedly, open chromatin is required for cross overs but the present data shows that all the euchromatin features are not positively correlated with crossovers and vice-versa. Most of our food crops are polyploids and the data availability is very limited (Bomblies 2023). So, it's a field waiting to be explored. Research on holocentric plants can give insights into chromatin features and their relation to recombination (Hofstatter et al. 2021). Studies with heterochromatin marks have been extensive and there are many euchromatin marks waiting for us to study their role in meiosis.

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Declarations

Conflict of interest We declare that we have no competing interest.

Consent for publication All authors consented for this publication.

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