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Epigenetic dynamics during sexual reproduction: At the nexus of developmental control and genomic integrity

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Abstract

Epigenetic marks influence gene regulation and genomic stability via the repression of transposable elements. During sexual reproduction, tight regulation of the epigenome must take place to maintain the repression of transposable elements while still allowing changes in cell-specific transcriptional programs. In plants, epigenetic marks are reorganized during reproduction and a reinforcing mechanism takes place to ensure transposable elements silencing. In this review, we describe the latest advances in characterizing the cell-specific epigenetic changes occurring from sporogenesis to seed development, with a focus on DNA methylation. We highlight the epigenetic co-regulation between transposable elements and developmental genes at different stages of plant reproduction.

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Keywords

Plant sexual reproduction, DNA methylation, Histone modifications, sRNA, Developmental genes, Sporogenesis, Gametogenesis, Seed development.

Abbreviations

TE, Transposable Element; VC, Vegetative Cell; SC, Sperm Cell; PGC, Primordial Germ Cell; PMC, Pollen Mother Cell; MMC, Megaspore Mother Cell; RdDM, RNA-directed DNA Methylation; sRNA, small RNA; CC, Central cell; EC, Egg cell; DAP, Days after pollination; FACS, Fluorescence-Activated Cell Sorting; INTACT, Isolation of Nuclei Tagged in specific Cell Types.

Introduction

Epigenetic marks have a strong influence on the expression of the genome, and its stability due to their role in silencing transposable elements (TEs). During their lifetime, organisms are adapting their epigenome to fulfill their transcriptional requirement. To ensure the erasure of marks from one generation to the next, the mammalian genome is going through two epigenetic resetting events during reproduction [1]. Plants do not seem to go through a similar resetting, but rather dynamic changes of epigenetic marks are taking place in different cell types involved in the reproductive process.

Plant reproduction starts with sporogenesis. It is initiated by the differentiation of a sporophytic cell into either pollen mother cell (PMC) or megaspore mother cell (MMC) and leads to the formation of the male haploid microspores and the female haploid megaspore. Following meiosis, during gametogenesis, male and female spores go through rounds of mitotic division to form the gametophytes. The male gametophyte, or pollen grain, is formed of two identical sperm cells (SC) and one large vegetative cell (VC). The female gametophyte contains seven cell types, including the two female gametes: the central cell (CC) and the egg cell (EC). During double fertilization, one SC fertilizes the EC and gives rise to the embryo, while the second one fertilizes the CC and gives rise to the endosperm [2]. During the different phases of plant reproduction, epigenetic marks, notably DNA methylation, are fluctuating.

In plants, DNA methylation can be found in three different cytosine contexts: CG, CHG, or CHH (where H is A, T, or C) [3]. Methylated cytosines can be faithfully copied on the newly synthesized strands during DNA replication by the DNA methylation maintenance system. Additionally, DNA methylation can be added *de novo* on unmethylated cytosines targeted by sRNA, involving a pathway called RNA-directed DNA methylation (RdDM). DNA methyltransferases are enzymes that catalyze the addition of a methyl group on cytosines, and different DNA methyltransferases are associated with different DNA methylation contexts and mechanisms. In the model plant, *Arabidopsis thaliana*, METHYLTRANSFERASE 1 (MET1) is the main DNA methyltransferase for the maintenance of CG methylation, CHROMOMETHYLASE 2 (CMT2) and CHROMOMETHYLASE 3 (CMT3) for the maintenance of CHG

and CHH and DOMAIN REARRANGED METHYLTRANSFERASE 2 (DRM2) for the *de novo* DNA methylation in all contexts mediated by the RdDM pathways. DNA methylation marks can also be removed through a mechanism of DNA cut and repair by DNA glycosylases, like DEMETER (DME) or REPRESSOR OF SILENCING 1 (ROS1). Chromatin remodelers are also involved in DNA methylation pathways such as DECREASED DNA METHYLATION 1 (DDM1), required for CG methylation [4], or the CLASSY proteins (CLSY1-2-3-4) required for CHH methylation [5].

In this review, we describe recent findings concerning epigenetic changes during reproduction, with a focus on DNA methylation and sRNA-related regulations. Although mentioned in parts, we will not develop in detail the changes of histones and their marks occurring during reproduction [6,7]. Recent data highlight the common epigenetic regulation of developmental genes and TEs during reproduction.

Main text

Epigenetic changes during sporogenesis

Sporogenesis is one of the key moments of the angiosperm life cycle as it allows the change from sporophytic to the gametophytic phase of the life cycle. Akin to mammalian Primordial Germ Cells (PGC), the PMC and the MMC differentiate, respectively from sporophytic tissue of the anther (male) and the placenta (female). In mammals, a reprogramming event takes place during PGC development involving a global loss of DNA methylation [1]. In plants, such a methylome erasure does not seem to occur, but several epigenetic changes have been observed during sporogenesis, which might provide plasticity to plant epigenetic inheritance.

Methylome data of *Arabidopsis* PMC (also called meiocyte) have become available through hand dissection [8]. PMC were shown to have lower CHH methylation, and higher CG and CHG methylation compared to sporophytic tissues. When this decrease in CHH methylation occurs during sporogenesis is still unsure. Observations using *in vivo* reporters for DNA methylation suggest that CHH methylation starts decreasing after meiosis [9], however, a decrease was already observed in PMC methylome data [8]. If we set aside possible experimental problems inherent to both techniques, CHH methylation could go through two successive changes: one occurring before meiosis and one in microspore.

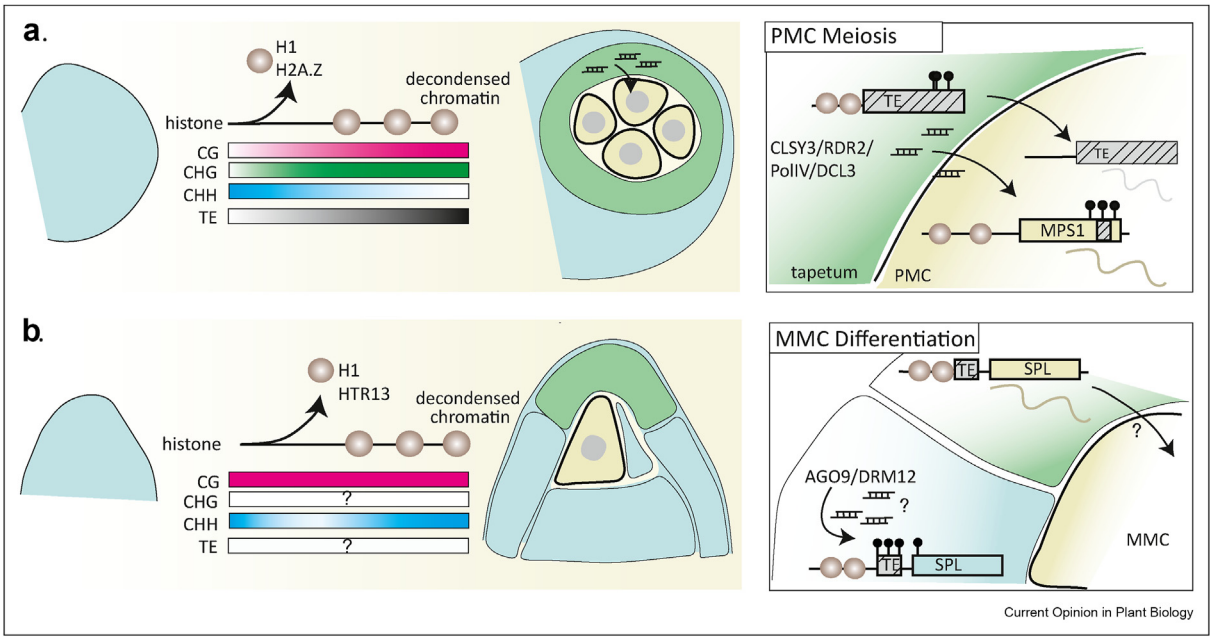
In addition to DNA methylation changes, the chromatin of PMC was shown to be globally decondensed compared to adjacent tissue [10]. This decondensation is accompanied by a loss of linker histone H1, loss of the histone variant H2A.Z, and changes in chromatin marks. PMC transcriptome data show that chromatin decondensation is associated with the expression of TEs, especially the ones located in centromeric and pericentromeric regions [11]. Similar changes occur during the differentiation of the MMC. During MMC specification, eviction of H1 and HTR13 histones and decrease of CHH methylation was observed in parallel to the decondensation of heterochromatin [9,12,13]. However, in this case, TE reactivation was not highlighted in transcriptomic studies [14].

Interestingly, communication occurs between sporophytic tissues and the PMC. During male sporogenesis, despite global CHH reduction, certain loci were found to be hypermethylated specifically in sexual cells (469 loci). These loci encompass Tes as well as coding genes. The proportion of developmental genes within these remains to be determined. Using FACS (Fluorescence Activated Cell Sorting) of tapetum cells and hand dissection of PMC, Long et al. recently showed that 24 nt sRNA produced in tapetum cells surrounding the PMC are responsible for the hypermethylation of those specific Tes via a non-cell-autonomous RdDM [15]. In addition to TE silencing, this phenomenon also regulates the expression of protein-coding genes (Figure 1a). Indeed, the hypermethylation situated in the gene *MULTIPOLAR SPINDLE 1* (*MPS1)* was found to regulate its splicing and consequently functionality. The *MPS1* gene is important for proper meiosis progression and therefore meiotic defects were observed in the RdDM mutants, *rdr2*, and *drm12* [16]. Independent studies have also shown the expression and function of two other members of the RdDM pathway: *ARGONAUTE 9* (*AGO9)* and *ARGONAUTE 4 (AGO4)* during male meiosis [17–19]. A similar communication between sporophytic tissue and MMC has been hypothesized. Defects in proper MMC specification have been observed in several mutants of the RdDM pathway including *ago9*, *ago4*, *ago6*, *pol-IV*, *rdr2*, *dcl3*, and *drm12* [20–22]. DRM2 and AGO9 regulate *SPOROCYTELESS* (*SPL*) needed for MMC specification (Figure 1b) [22]. Interestingly, a similar phenotype is also present in mutants affecting CG methylation and the trans-acting sRNA pathway suggesting a very complex regulation of MMC specification [23,24]. However, due to the lack of cell-specific genome-wide data, it is difficult to conclude yet on the nature of the non-cell-autonomous signal. Despite some similarities, the epigenetic reprogramming occurring during PMC and MMC differentiation might differ.

Acquisition of cell-specific epigenome during gametogenesis

In recent years, epigenomic modifications occurring during male gametogenesis have been the subject of several cell-specific genome-wide approaches. In opposition to female gametogenesis, where every stage of development is tightly embedded in sporophytic tissue, male gametogenesis ends with the release of free pollen grains which facilitate the extraction of each cell type. The first epigenomic data sets were obtained

Figure 1



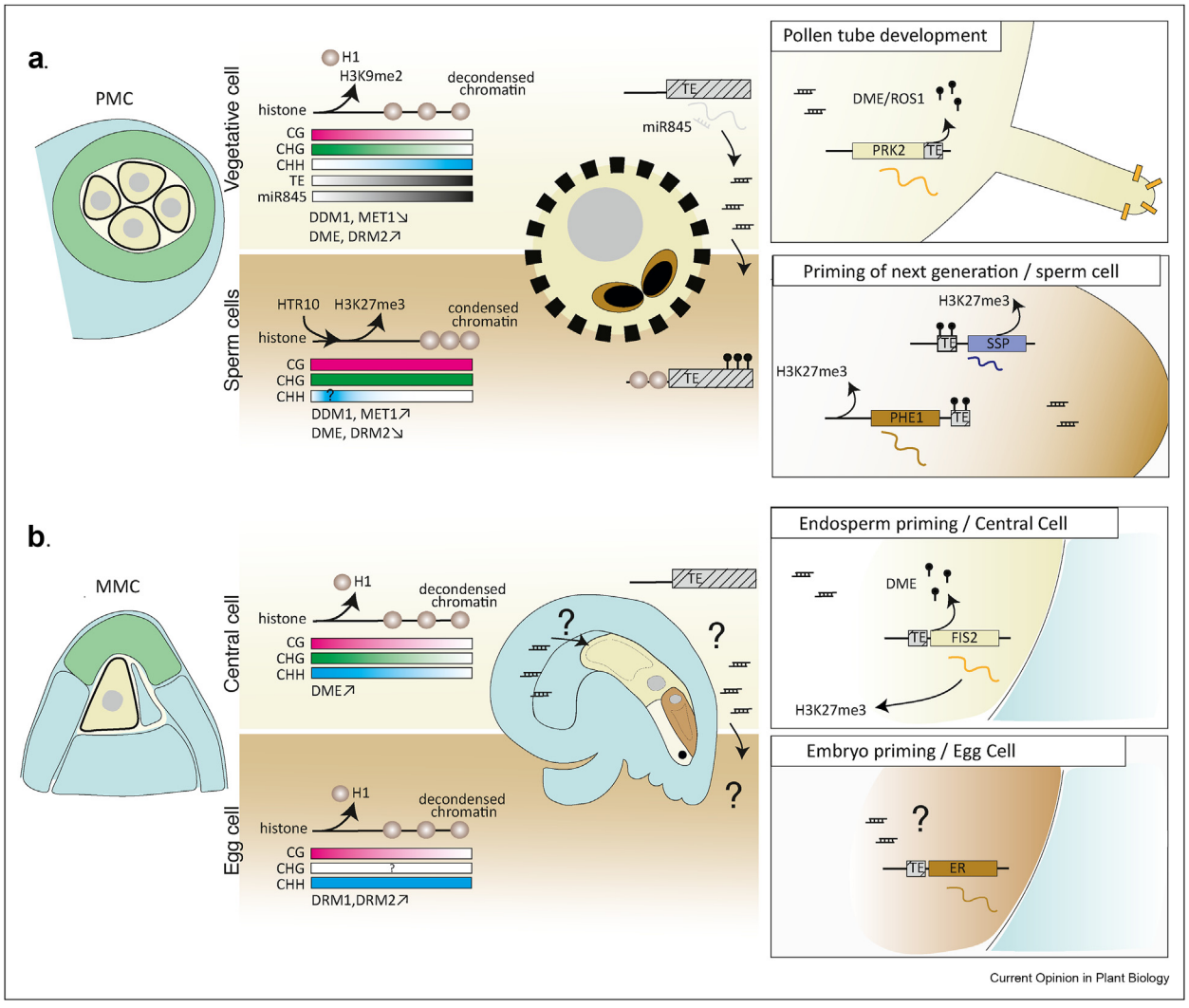
Schematic representation of epigenetic dynamic during *Arabidopsis* male (**a**) and female (**b**) sporogenesis. Spheres represent nucleosomes. Major changes in histone modifications, histone variants, and histone linker H1 are indicated. CG methylation, CHG methylation, CHH methylation, and TEs activity are represented by magenta, green, blue, and gray gradients, respectively. The gradients represent the changes occurring during the specification of the PMC (**a**) and MMC (**b**). DNA methylation data during male sporogenesis rely on methylome data while data during female gametogenesis rely on methylation sensors. On the left, examples of the regulation of developmental genes are illustrated. (**a**) In PMC, *MULTIPOLAR SPINDLE 1* (*MPS1)* is required for proper meiosis and controlled by RdDM-dependent splicing which relies on TE sRNAs produced in the tapetum. The production of these tapetum sRNAs relies on CLASSY3 (CLSY3), RNA-DEPENDENT RNA POLYMERASE 2 (RDR2), RNA POLYMERASE IV (POLIV), and DICER 3 (DCL3) [16,84]. (**b**) MMC differentiation requires *SPOROCYTELESS* (*SPL*) expression in the apical nucellar cell (green) which is adjacent to the MMC (yellow). In other surrounding cells (blue), *SPL* is silenced by the RdDM pathway [22].

from mature pollen where the sperm cells (SC) and the vegetative cell (VC) nuclei could be separated using FACS and cell-specific fluorescent marker lines. Methylome analysis from isolated SC in *Arabidopsis* revealed that CG and CHG methylation are mostly stable in the male germline while CHH methylation is lower compared to aerial tissues [25]. Low CHH methylation in the male germline seems relatively stable as it is also detected earlier in PMC. The absence of large DNA methylation changes during SC differentiation likely participates in the stable inheritance of DNA methylation pattern to the next generation observed in *Arabidopsis*.

As opposed to SC, the VC is displaying changes in DNA methylation during pollen gametogenesis: a gain of CHH methylation and a decrease of CG and CHG. This demethylation involves the eviction of linker histone H1 and is associated with a gain of chromatin accessibility [26,27]. These changes are correlated with the high expression of DRM2 and DME but low expression of MET1 and DDM1 in the VC compared to SC [25,28–30]. Interestingly, *dme* pollen grains were shown to have defects in paternal transmission suggesting a role of VC *dme* demethylation in proper VC function [30]. Recently, such a hypothesis was confirmed in a study showing that both *ROS1* and *DME* demethylation activity in the VC is necessary for the activation of genes controlling pollen tube function [31]. Out of 194 genes annotated to be involved in pollen tube function, 108 of these genes show VC hypomethylation. The overlap with TE remains to be analyzed. Some of these genes were found to have TE in their vicinity, for example, *POLLEN RECEPTOR LIKE KINASE 2* (*PRK2*) (Figure 2a). In addition to such an effect on developmental genes, VC demethylation principally affects TEs and leads to their expression in the VC. Reprograming of the VC methylome appears to be initiated during the first mitosis of male gametogenesis when SC and VC identity are specified. Recently, it was shown that a similar contrast between SC and VC DNA methylation is also found in other plant species like rice [32], or tomato [33] suggesting its conservation in plants.

Similarly, to the VC, DME activity was shown to be essential for the CC function in the female gametophyte [34,35]. Loci-specific demethylation in the CC is tightly linked to the expression of imprinted genes which are

Figure 2



Schematic representation of epigenetic reprogramming during *Arabidopsis* male (**a**) and female (**b**) gametogenesis. Spheres represent nucleosomes. Major changes in histone modifications, histone variants, and histone linker H1 are represented such as H3K27me3 eviction in sperm cells. CG methylation, CHG methylation, and CHH methylation are represented by magenta, green and blue gradients, respectively. TEs activity and miR845 expression are represented in black gradients. Significant expression changes in DNA methylation regulators are indicated for each cell type. The gradients represent the changes occurring during male (**a**) and female (**b**) gametogenesis. DNA methylation during male gametogenesis relies on methylome data while during female gametogenesis gradients rely on methylation sensors. On the left, examples of the regulation of developmental genes are illustrated. (**a**) miR845 triggers the accumulation of 21 & 22 nt TE sRNAs in the VC which move to the SC to promote TE regulation by canonical RdDM [66,85]. In the VC, demethylation of the *POLLEN RECEPTOR LIKE KINASE 2* (*PRK2*) gene by *DME* is necessary for pollen tube development [31]. In sperm cells, H3K27me3 eviction allows the activation of paternally expressed genes such as PHERES1 (*PHE1)* or *SHORT SUSPENSOR* (*SSP)*. (**b**) In the CC, *DME* activity is required to activate maternally expressed genes such as *FERTILIZATION INDEPENDENT SEED 2 (FIS2)*. Due to the absence of data concerning the EC, we can only hypothesize that *ERECTA* (*ER*) expression could be primed in the EC resulting in maternal control of early embryo development [86].

important for proper endosperm development after fertilization [36]. However, unlike the VC, linker histone H1 seems to have an antagonist effect to DME in the CC where an H1 mutation leads to increased DNA methylation on imprinted genes [37]. Additionally, DME also targets TEs in the CC, like in the VC. Methylome analysis revealed that the CC is hypomethylated compared to sporophytic tissue, in part due to DME activity [38]. General hypomethylation is also observed in the rice CC [38]. Interestingly, unlike the VC, the CC does not show an increase in CHH methylation suggesting that two different mechanisms take place in both accessory cells.

The methylome of the *Arabidopsis* egg cell (EC) is currently unknown but observation of *in vivo* DNA

methylation reporters suggests that CG methylation is reduced in the EC compared to surrounding tissue while CHH methylation is stable [9]. The methylome of rice EC is rather hypermethylated compared to other tissues [38]. Despite this global hypermethylation, three DNA demethylases (DNG702, DNG701, and DNG704) have recently been shown to demethylate specific regions in the rice EC, showing that loci-specific DNA demethylation takes place in the rice EC. This DNA demethylation ensures the expression of reproduction-related genes, consequently, *dng702* mutants fail to start embryogenesis after fertilization [39]. The investigation of the dynamics of DNA methylation changes during female gametogenesis has been hindered by the difficulty to access those embedded cell types. The observation of live reporters of DNA methylation suggests that no major changes in global DNA methylation occur during female gametogenesis. However, methylome analyses of functional megaspores and different stages of female gametophyte development are currently lacking.

In addition to the changes in DNA methylation, pollen cell differentiation also involves changes in histone repertoire and histone marks. In SC, the alternative histone variant H3.10 is gradually incorporated into the SC chromatin after the first pollen mitotic division [40]. The H3K27me3 methylation mark is erased in mature sperm cells [41,42] partly due to H3.10 incorporation [41]. The erasure of H3K27me3 participates in the activation of SC-specific genes (Figure 2a). In parallel, a significant loss of the H3K9me2 methylation mark has been observed in the VC, as well as loss of linker histone H1 [26,43]. ATAC-seq at different pollen developmental stages has shown an open chromatin state of the constitutive heterochromatin, especially in pericentromeric TEs associated with their transcriptional reactivation in VC [27]. In the female gametophyte, a clear decondensation of the chromatin is observed compared to somatic tissues. It is accompanied, like in the VC, by a loss of histone H1 in both EC and CC [9,44]. However, the detection of H3K9me2 in CC and EC again suggests that the regulation of epigenetic marks might not be mirrored between male and female gametogenesis [45].

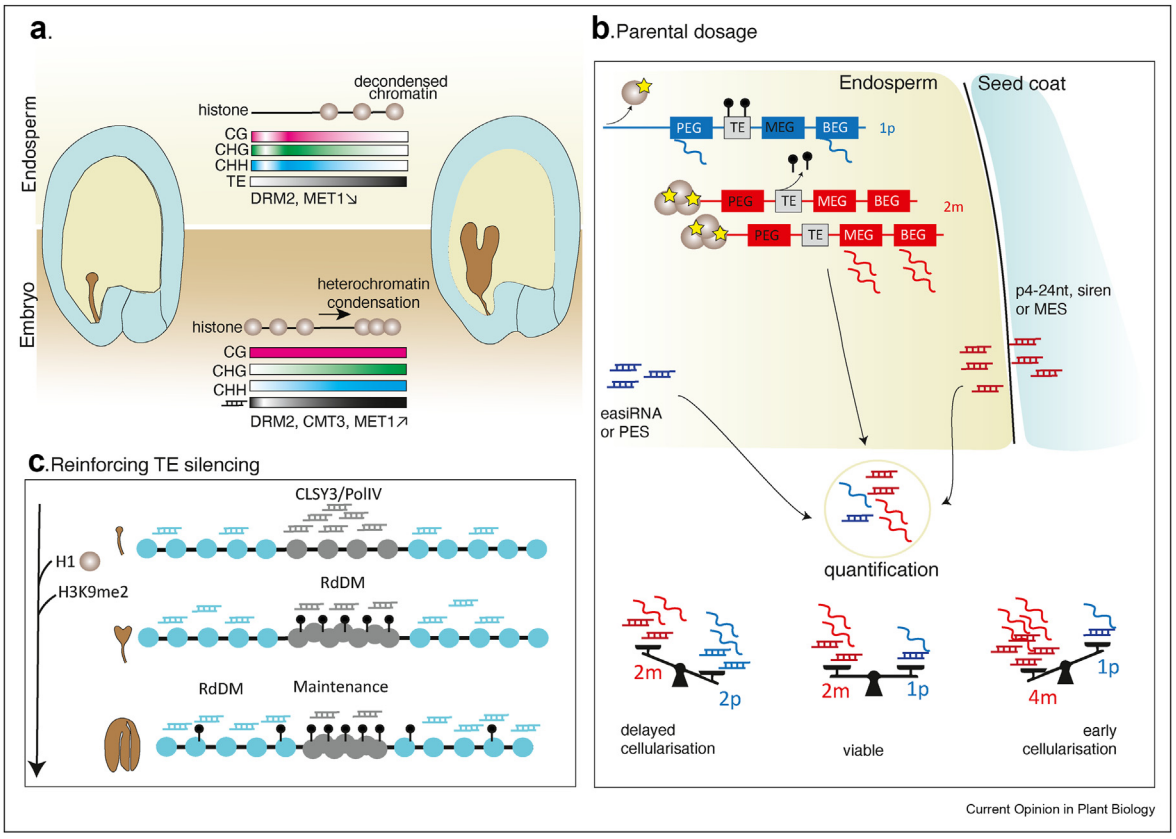
The general decondensation of constitutive heterochromatin in the VC during pollen development results in an increased expression of TEs and associated small RNAs in the VC [26,27,29,43]. Those sRNAs have been shown to move from the VC to the SC to reinforce TE silencing in the SC and likely participate in maintaining the genomic stability of the next generation [29,46,47]. A similar non-cell-autonomous phenomenon was proposed to occur also in the female gametophyte (Figure 2b) [48]. Indeed, sRNAs have been shown to move from the CC to the EC [48–50]. Additionally, very abundant maternally expressed sRNA, (likely originating from the ovule and seed integuments) called siren sRNA were found in several species [51] including *Arabidopsis* where their biogenesis relies on CLSY3 and PolIV [52]. However, the evidence for their role or sRNA movement in TE silencing in the EC remains to be demonstrated.

Epigenetic reinforcing during seed development

Similarly, to sporogenesis and gametogenesis, dynamic changes in the epigenome have been observed during seed development. Interestingly, the endosperm and embryo lineage are going through very contrasted epigenetic changes during their development. The late endosperm (7-9 DAP) displays a significantly lower level of DNA methylation in all contexts compared to the embryo, especially the maternal genome, and chromatin decondensation [48,53–55]. This loss of DNA methylation is dependent on DME activity in the central cell, before fertilization [38,48,53,54]. However, DME activity in the CC is only partially the cause of endosperm hypomethylation as DNA methylation is lower in late endosperm than observed in the central cell suggesting that other factors could contribute to this difference, such as the low expression of DNA methyltransferases in the endosperm [38,56]. Interestingly, methylome analysis on INTACT sorted endosperm nuclei at 4 DAP and 6 DAP revealed an even lower DNA methylation level than in the CC or late endosperm, suggesting that DNA methylation might decreases further after fertilization but increase later during endosperm development [57]. A deeper analysis of the DNA methylation changes using the methylome data obtained in those different studies as well as earlier time points during endosperm development would allow us to clarify the extent of these changes. Interestingly, epigenetic changes might also not occur uniformly in the endosperm as differences in TE and imprinted genes expression were observed between endosperm domains [58–60].

Endosperm DNA methylation influences imprinted gene expression [36,61]. Imprinted genes are often associated with TE sequences, *DME* demethylation, and for some genes also with H3K27me3 methylation mark [62,63]. Imprinted gene expression is thought to be at the heart of the regulation of parent-of-origin effects on endosperm development such as the regulation of parental genome dosage (Figure 3). Failure in this regulation results in defective endosperm development ultimately leading to seed abortion. In addition to DNA methylation, RdDM components and sRNA were also shown to be involved in parental genome dosage [64–70]. Several studies have especially highlighted the importance of the parental inheritance of sRNA in this process. These regulations seem conserved across plant species as similar regulations were found in *Brassica rapa* [71,72] and rice [73,74]. Ultimately, the parent-of-origin nature of such phenomenon implies an inheritance or priming from the parents either genomically (e.g. DNA methylation marks) or cytoplasmically (e.g. sRNA).

Figure 3



Schematic representation of epigenetic dynamic during *Arabidopsis* seed development. (**a**) In endosperm, the chromatin remains decondensed while the chromatin is recondensing in the zygote during seed maturation. CG, CHG, and CHH DNA methylation are represented by magenta, green and blue gradients, respectively. sRNA increase in the embryo is represented by a black gradient. The gradients represent the changes occurring during seed development aggregating data from methylome and methylation sensors. The time window for endosperm data is 4–8 days after pollination while it is pre-globular to mature green for the embryo data. (**b**–**c**) Examples of epigenetic regulations taking place in the endosperm (**b**) and the embryo (**c**) are illustrated. (**b**) Parental dosage is strongly influenced by the expression of imprinted genes during endosperm development. Changes in the 2m:1p dosage lead to endosperm developmental defects. Paternally Expressed Genes (PEG) on the maternal genome are silenced by H3K27me3 marks (yellow stars) while Maternally Expressed Genes (MEG) are silenced by DNA methylation on the paternal genome. Parental dosage is also regulated by sRNAs such as paternal easiRNA that accumulate in pollen before fertilization and P4 24 nt or siren siRNA produced maternally in the endosperm and/or the seed coat. We propose to generally abbreviate them as Paternally Expressed sRNAs (PES) and Maternally Expressed sRNAs (MES). (**c**) TE silencing reinforcing occurs during embryogenesis. Chromatin decompaction, siRNA abundance, and the expression of RdDM components allow *de novo* methylation of centromeric and pericentromeric regions. Gradual compaction, allowed by the incorporation of linker histone H1, and stable remethylation of centromeric regions displace RdDM activity toward chromosome arms later during embryogenesis.

In Arabidopsis, unlike the endosperm, the embryo displays more CG, CHG, and CHH methylation compared to aerial tissue [53]. The lower CG DNA methylation detected in EC using DNA methylation sensors is recovered quickly after fertilization [9]. Embryo methylomes at different stages of development have revealed that CHH DNA methylation is increasing during embryogenesis, reaching a level superior to mature tissue [56,75–77]. Such an increase in CHH methylation is also observed during soybean seed development [78]. It is often referred as a reinforcing mechanism to ensure the proper silencing of transposons in the new generation. The increase of DNA methylation in the embryonic lineage has not been linked to the expression of genes involved in embryo development. Increased CHH methylation is relying on strong RdDM activity in the embryo as well as the presence of a large amount of 24 nt sRNA in the early embryo [56,75]. It has been hypothesized that those sRNA could originate from the hypomethylated endosperm [77]. However, the physical isolation of the embryo from the endosperm occurring from the globular stage does not support this hypothesis at least during later stages of seed development [79]. It was recently shown that distinct sRNA populations corresponding to TEs are detected during embryogenesis [75]. Interestingly, two clusters with different

dynamics were identified: one corresponding to rather centromeric heterochromatic TE and the other to pericentromeric euchromatic TE. This study suggests that the decondensation of the centromeric heterochromatin, possibly linked to H1 depletion, at the early stages of embryo development leads to the production of 24 nt sRNA at the centromeres likely to initiate *de novo* DNA methylation and guide H3K9me2 at those sequences (Figure 3) [75,80]. The centromeric constitutive heterochromatin is later repressed by the MET1/CMTs/H3K9me2 machinery [75]. The initial sRNA population in the EC and the zygote remains to be determined in *Arabidopsis* to evaluate the potential role of parental and/or moving sRNA at the early stages of embryo development. A recent study in rice showed that the sRNA population is very rapidly changing from the gametes to the one-cell zygote, but a maternal carryover of siren sRNA is observed [81].

Conclusion

In this review, we highlighted recent reports investigating the reorganization of epigenetic marks that are occurring at various stages of plant reproduction, from sporogenesis to seed development. Some obstacles linked to the isolation of some cell types remain to be overtaken, especially for female sporogenesis and gametogenesis as well as early seed development. Further studies will allow us to better understand how the epigenome is maintained through generation as well as its potential modification. Interestingly, studies have highlighted an interesting link between the expression of developmental genes and the changes in epigenetic marks occurring during reproduction. Developmental genes appear to be coregulated by the epigenetic reprogramming initially linked to TE defense. Conversely, the epigenetic reprogramming main role could be to ensure proper development and the reactivation of TE be only a consequence of those epigenetic changes. The cohabitation of each aspect has likely been selected during angiosperm evolution and models for the involvement of TEs in the evolution of gene networks have been proposed [82,83].

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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