Chapter 6
Setting the Stage for the Next Generation: Epigenetic Reprogramming During Sexual Plant Reproduction

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Introduction

Every multicellular organism consists of groups of similar cell types (tissues) that are specialized to carry out distinct functions. Cell differentiation is a product of a gradual commitment of a totipotent cell which is capable of developing into all the specialized cells that make up the adult organism in the course of development. The union of a sperm and an egg, two highly differentiated cells, generates a self-contained entity, a totipotent zygote, that has the ability to produce lineages of pluripotent cells that are predestined to a particular cell fate (cell fate specification) which in turn will become highly differentiated cell types (cell fate determination).

As every cell contains the same genomic information, the process of cell differentiation must reflect highly controlled modifications of gene expression. Over the past few years, we have begun to understand how cell-type-specific expression patterns and the process of cell specification seem to be determined or regulated by reversible epigenetic changes which are gradually imposed on the genome during development. These epigenetic modifications can be accomplished in different ways (DNA methylation, histone modification, chromatin remodelling and using the small RNA machinery), be inherited across generations and determine parent-of-origin-specific patterns of inheritance (genomic imprinting) [1].

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In plants, every cell has the ability to reversibly dedifferentiate and become totipotent, contrasting with animal cells that become committed earlier during embryogenesis at 2–4 cell stage [2]. Several examples occurring in the plant adult stage are illustrative of the remarkable plasticity of plant cells. The plant life cycle in flowering plants is marked by multiple developmental transitions [3] involving the determination of new cell fates: (A) reprogramming of the apical meristem from a vegetative to a reproductive fate; (B) reprogramming of floral somatic cells to a germ cell lineage (pluripotency); (C) differentiation of specialized female and male gametes and (D) fertilization and restoration of a totipotent zygote.

While the epigenetic state of plant cells is thought to be relatively stable during development, epigenetic modifications occurring during germline specification and early embryogenesis are essential for gamete differentiation, re-establishment of pluripotency in the embryo and configuration of parent-specific epigenetic states. Epigenetic reprogramming through selective maintenance and erasure of epigenetic marks in the germline also plays a fundamental role determining other aspects such as inheritance of induced phenotypic traits and maintenance of genomic stability. The sessile nature of plants makes them to face multiple environmental perturbations during their life cycle that can cause physiological and developmental alterations or have consequences in genomic stability. Inducible phenotypical responses have been shown to affect profoundly the expression of genes potentially regulated by epigenetic marks [4]. Epigenetic modifications induced by stress are mitotically stable and if not reset they can be transmitted to the next generations through the germline (transgenerational epigenetic inheritance) [5–8]. Reduction of DNA methylation in *Arabidopsis thaliana* was shown to cause increased mortality, stunted growth, delayed flowering and lower seed set, supporting that epigenetic modifications contribute to increased plant fitness [9], but the contribution of induced epigenetic modifications in the evolution of natural populations is still a matter of debate [10]. Environmental stresses are also accompanied by an increase in transposon activity [4] and their mobilization can have adverse effects, generating deletions, genomic rearrangements and causing gene misregulation, ultimately compromising genome integrity and stability [11]. More rarely, mutations arising from transposon activity can generate genetic variation that may allow plants to adapt to adverse environments [12]. A strict control of transposon activity is thus imperative to protect the genome from potential deleterious effects across generations, especially in plants, where the germline is specified late during development. An understanding of how plant cells reprogramme themselves, the regulatory circuitry that maintains their ability to become totipotent or pluripotent and the events that commit progenitor cells to particular differentiation states are of paramount importance. In this chapter, we summarize and discuss recent studies into the epigenetic reprogramming occurring during one of the most dramatic phase transitions in the plant life cycle, the transition from a diploid to a haploid phase (sporophytic-to-gametophytic phase transition) which leads to germline specification and the renewal of the plant life cycle through fertilization and re-establishment of pluripotency in the embryo.

Epigenetic Control of Transition to Reproductive Development

Flowering plants spend most of their life cycle in a vegetative phase (diploid sporophytic generation) and the transition to a reproductive phase occurs when the plant reaches maturity. The timing when this developmental transition takes place is of utmost importance to ensure reproductive success, since flowering should occur in favourable physiological and environmental conditions to assure the completion of the fertilization process and dispersal of seeds.

The transition to flowering is a significant developmental change in the plant life cycle and is dependent on complex genetic pathways that integrate information from endogenous factors (hormone and nutrients) and environmental cues such as day length (photoperiod) and temperature (vernalization) [13]. In recent years, it has become clear that the genetic pathways controlling important phase transitions, namely juvenile-to-adult phase and the transition to a reproductive phase, share some common regulatory factors [14, 15]. Moreover, the expression of many of the different flowering genes involved in these phase transitions seems to be regulated by epigenetic modifications, alternative splicing, antisense RNA and chromatin silencing [3, 16–18]. In *Arabidopsis*, the expression of *FLOWERING LOCUS C* (*FLC*), a *MADS*-box transcription factor, is responsible for the direct repression of flowering pathway integrators *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). A number of chromatin-modifying components seem to be involved in the repression of *FLC* expression [19]. In response to cold, the upstream component of the vernalization pathway, a chromatin remodelling plant homeodomain (PHD) finger protein, VERNALIZATION INSENSITIVE 3 (VIN3), binds to FLC chromatin [20] interacting with components of the polycomb-group repressive complex 2 (PRC2) to mediate *FLC* silencing through histone H3K27 tri-methylation (H3K27me3) [21, 22], which is essential to reinforce and stabilize a stable epigenetic memory of vernalization through mitotic divisions [23]. Moreover, *FLC* antisense transcripts negatively regulate *FLC* sense transcription by triggering localized histone H3K4 demethylation [24, 25]. In addition, two evolutionary highly conserved microRNAs, *miR156* and *miR172*, and their respective target genes (*SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* and *APETALA 2* transcription factors) were also implicated in the regulation of flower transition (reviewed in 3).

In order for plants to be able to respond to vernalization and prevent early flowering, some type of reprogramming process is likely needed to erase the effects of repressive modifications in FLC chromatin leading to flowering. Independent from the epigenetic state of the maternal plants, *FLC* seems to be temporarily reactivated in male and female reproductive tissues (anther tapetum and ovule integuments) but is repressed in both male and female gametophytes. After fertilization, the paternal derived *FLC* copy is reactivated in the zygote and the maternal derived *FLC* copy is first expressed in the early multicellular embryo [26, 27], while no expression is detected in endosperm. This resetting could thus result from reprogramming associated

with histone turnover as is shown in the zygote (see below) or from local recruitment of H3K27me3 demethylases or specific chromatin remodelers after fertilization. The resetting of *FLC* expression during early embryogenesis thus seems to be a prerequisite for the repressive effect of FLC in flowering in the following generation.

In the plant apex, the integration of signals promoting flowering triggers the activation of flower meristem identity genes such as *LEAFY, APETALA 1* (*AP1*) and *CAULIFLOWER,* transforming the apical meristem into an inflorescence [28]. The activation of the five homeotic classes (ABCDE) of flower identity genes in different regions of the meristem and their combinatorial interaction determine the positioning of four different types of flower organs [29]: in the outer most whorl the sepals, then petals, the male reproductive organs (stamens and anthers, collectively known as androecium) and in the central whorl the female reproductive organ, consisting of one or more carpels that make up the gynoecium or pistil.

Specification of Gametophytic Cell Fate

In flowering plants, differentiation of plant gametes is an event occurring late during flower development. In contrast to animals where gametes are directly specified from meiotic products, plant gametes result from a post-meiotic developmental process that results in the formation of multicellular structures, the gametophytes, where gametes are coupled to accessory cells that facilitate gamete function [30]. A series of proliferative and cell specification events determine the formation of specialized reproductive organs that culminate with the differentiation of tissues and cells with reproductive and nonreproductive functions; however, at this stage no traceable germ cell lineage is yet specified.

Within the anther, an inner cell layer of secretory cells called tapetum supports microsporogenesis that initiates in a central core of cells, the sporogeneous pollen mother cells (PMC). The PMCs define the male reproductive cell lineage leading to the development of a male gametophyte, the pollen grain. At this stage in the anther, the PMCs are connected by enlarged cytomitic channels which create a cytoplasmic continuum thought to promote effective synchronization during meiosis. Two meiotic divisions transform each PMC into a tetrad of haploid and unicellular microspores (UNM); the cytomitic channels disappear, establishing an individual cytoplasm in each microspore, each with its own callose envelope and all encased in the callose wall of the tetrad. Pollen wall deposition initiates and an exine layer develops around each microspore. After the haploid microspores are released from the tetrads, important cytoplasmic and gene expression changes are believed to reset the sporophytic programme and launch a gametophytic programme (reviewed by [31]). Gametogenesis initiates with a reorganization of the microspore cytoplasm, whereby small vacuoles coalesce into a single vacuole that polarizes the nucleus to one side of the microspore. This cell polarization is determinant to establish the first asymmetric division called pollen mitosis I (PMI), in which the two daughter cells acquire different cell fates: the large vegetative cell (VC), the pollen grain,

enclosing a small generative cell (GC) [32] (Fig. 6.1a). Thus, only at the bicellular pollen (BCP) stage the germline, in the form of a generative cell, is clearly specified. It was proposed that the different cell fates are caused by polarized distribution of gametophytic regulatory factors during the asymmetric division, resulting in the repression of vegetative cell-specific genes in the generative cell [33]; however, the processes determining commitment to different cell fates are still poorly understood. The GC undergoes a second mitosis, pollen mitosis II (PMII) to produce two functional twin male gametes, the sperm cells (SC; Fig. 6.1a). Depending on the species, PMII can either take place before dehiscence or during pollen tube growth within the female tissues. The "cell within a cell" structural organization of the male gametophyte in angiosperms relies on an intimate association of the male gametes (sperm cells) with the vegetative nucleus (VN), called the male germ unit (MGU) [34] (Fig. 6.2). In this structural association, a membrane of vegetative origin encloses both sperm cells and extends from one of the sperm cells through a projection to the vegetative nucleus. Within the MGU, both sperm cells are transported as a unit by the leading VN until pollen tubes deliver the sperm cells in the vicinity of female gametes. While this endomembrane of vegetative origin may provide protection, isolating sperm cells from direct contact with the cytoplasmic environment of the pollen tube, it also establishes a possible communication route between the vegetative nucleus and sperm cells [35]. Moreover, the physical adherence of both sperm cells revealed by two sperm-specific tetraspanins (*TETs*) localizing in a membrane microdomain in the interface between both sperm cells may provide another level of communication [36] (Fig. 6.2). Intercellular interactions occurring within female tissues during pollen tube growth are essential for pollen tubes to acquire competence to sense female guidance signals [37], but they also seem to impact sperm cell behaviour as in some plant species sperm cells must undergo a process of maturation before they acquire fertilization competence [38]. These physical connections between the vegetative nucleus and sperm cells or between sperm cells may have functional implications in maintaining an equivalent fertilization competence of both sperm cells [39] and/or in maintaining germline identity within the pollen vegetative cytoplasmic environment. Ultrastructural studies will be essential to reveal the nature of these cellular connections.

Development of the female gametophyte takes place in the carpel. Here, rows of ovule primordia arise from the placental tissue along the margins of the site of carpel fusion, the septum. In each ovule primordium, within a supportive and nourishing tissue called nucellus, megasporogenesis occurs with the differentiation of the reproductive female lineage, the archeosporial cell. This cell differentiates into the megaspore mother cell (MMC), which undergoes meiotic reduction to give rise to four haploid megaspores (Fig. 6.1b). After meiosis, four haploid cells are surrounded by a very thin cell wall, with plasmodesmata connecting the chalazal megaspore to neighbouring nucellar cells. A positional signal is thought to promote cell death of three of the four megaspores. Megagametogenesis begins when the surviving functional megaspore goes through three rounds of mitosis to form first a two-nucleated, four-nucleated and subsequently eight-nucleated syncytium gametophyte. Different patterns of gametophyte development have been described

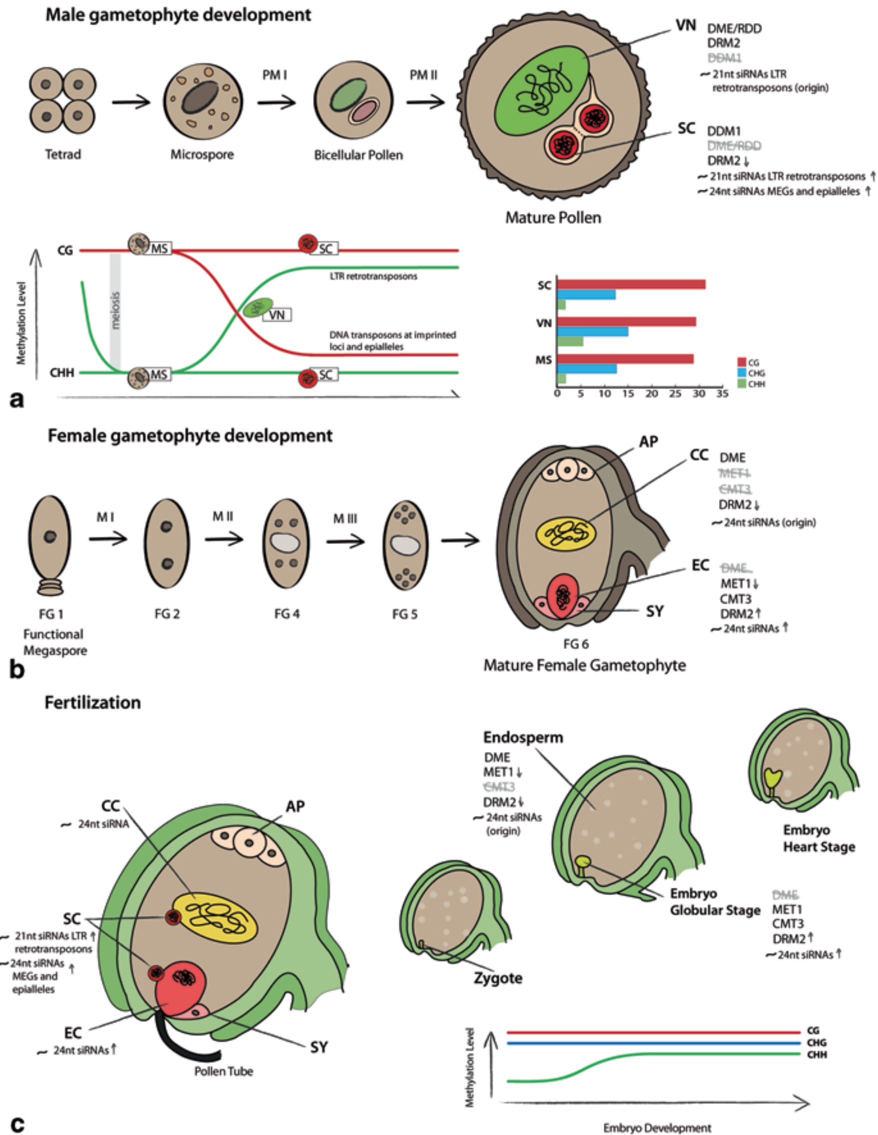

Fig. 6.1 Epigenetic reprogramming during gametophyte development, fertilization and embryogenesis. Sexual reproduction in *Arabidopsis thaliana* involves extensive epigenetic programming with far-reaching potential consequences for genome stability, imprinting and epigenetic inheritance. **a** During male gametophyte development, haploid microspores (MS) divide asymmetrically (PM I) to give rise to bicellular pollen consisting of a vegetative cell harbouring a smaller generative cell. The latter will undergo a second mitosis (PM II), originating two sperm cells (SC) that stay connected to the vegetative nucleus (VN) via a membrane projection. Microgametogenesis is characterized by a loss of CHH methylation from LTR retrotransposons in microspores and sperm cells due to reduced expression of *DRM2*, while it is restored in the vegetative nucleus. Missing expression of the chromatin remodeler *DDM1* in the vegetative nucleus however leads to the

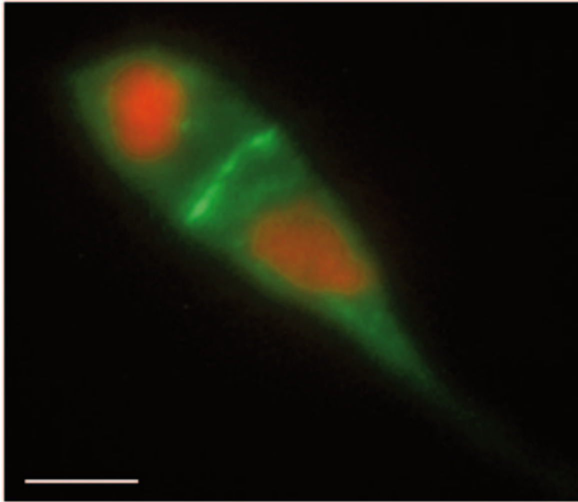

Fig. 6.2 Sperm cell connection in the male germ unit. *TET12p*:TET12-GFP and *HTR10p*:HTR10-mRFP protein fusions expressed in sperm cells within an *in vitro* germinated pollen tube. GFP fluorescence highlights the membrane microdomain connection between sperm cells within the male germ unit; mRFP fluorescence corresponds to nuclear localization of the male germline specific histone H3.3 variant (HTR10). Scale bar: 2 µm

in several species, differing from each other by variations on cytokinesis during meiosis, on the number of mitotic divisions and in the cellularization pattern. Cellularization and the final differentiation of a *Polygonum*-type female gametophyte

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activation of *ATHILA* retrotransposons and accumulation of correspondent 21-nt siRNAs in the sperm cells. Moreover, the loss of expression of the DNA glycosylases *DME/RDD* in the vegetative nucleus affects CG methylation of transposons neighbouring imprinted loci and epialleles, with correspondent 24-nt siRNAs accumulating in the sperm cells. PM I pollen mitosis I, PM II pollen mitosis II, MEG maternally expressed gene, ↓ down-regulation, ↑ up-regulation, ~~Gene~~ not expressed. **b** During female gametogenesis, the functional megaspore undergo three consecutive mitosis (M I–III) to generate an eight nucleated cell (FG5). Subsequent cellularization and differentiation results in a seven-celled embryo sac (FG6) consisting of two female gametes, the diploid central cell (CC) and the haploid egg cell (EC) with two types of accessory cells, three antipodals (AP) and two synergids (SY). In the CC, the loss of expression of the methyltransferases *MET1* and *CMT3* and the downregulation of *DRM2* together with high expression of *DME* generate a hypomethylated state. The resulting transcriptional activation of transposable elements leads to the production of 24-nt siRNAs that accumulate in the adjacent egg cell, where RNA-directed DNA methylation (RdDM) pathways are active (*DMR2* expression). FG (female gametophyte) stages according to the classification described in [128]. **c** Upon double fertilization, one sperm cell fuses with the egg cell and the second with the central cell, giving rise to the diploid embryo and the triploid endosperm, respectively. Each gamete carries genetic and epigenetic information, e.g. hypomethylated transposon sequences and siRNAs that create a condition of epigenetic reconciliation upon fusion. The epigenetic state of the endosperm resembles the one of the central cell before fusion with low levels of methylation and production of 24-nt siRNAs. The accumulation of those 24-nt siRNAs in the embryo will participate, possibly together with 24-nt siRNAs delivered by the sperm cells, in *DRM2*-driven RdDM to restore CHH marks during embryo development. SN (sperm nucleus)

involve the specification of four cell types with distinct functions in a seven-celled embryo sac (ES): two gametes, consisting of a haploid egg cell (EC) and a diploid central cell (CC), and two accessory cell types consisting of two synergids (Sy) and three antipodals (AP) ([31] and references therein) (Fig. 6.1b).

Methylation Reprogramming During Pollen Development in *Arabidopsis*

Much of our current understanding of epigenetic modifications during germline specification in plants comes from the terminal developmental stages of gametophytes, but there are growing evidences that epigenetic modifications might be relevant during somatic-to-reproductive transition. These epigenetic changes can have a significant impact in the next generation, as the transition from a mitotic to a meiotic cell cycle programme sets the stage whereby a group of cells acquire a sporogenous reproductive cell fate. The differentiation of PMCs is intimately linked with the differentiation of surrounding tissues in the anther. Modifications in microRNA activity, chromatin remodelling and DNA methylation play a critical role in the differentiation of anther tissues and in tapetum-programmed cell death [40–42]. How these epigenetic signals contribute to differentiation of microspores is still poorly understood, but evidences showing that *trans*-acting 24-nucleotide (nt) short interfering RNAs (siRNAs) produced in anther tapetum can impact male gametophyte development support this hypothesis [43].

The nature of the switch that defines the mitotic to meiotic transition is still unknown, but DNA methylation, histone modifications and siRNAs seem to be involved in this cell fate transition. DNA METHYLTRANSFERASE 1 (MET1) and DECREASE IN DNA METHYLATION 1 (DDM1) are important for meiotic progression and pattern the recombination frequency along chromosomes [44–47]. In rice, the germline specific *MEIOSIS ARRESTED AT LEPTOTENE 1* (*MEL1*), a homologue of *Arabidopsis ARGONAUTE 5* (*AGO5*), is required for mitosis in sporogeneous PMCs and meiotic progression, but does not affect specification of the reproductive cell lineage [48]. However, the *Arabidopsis ago5* knock-out does not show any discernible phenotype [49], suggesting that in *Arabidopsis*, *AGO5* may act redundantly with other *AGO* members.

Transcriptomic analyses of plant meiocytes are relative recent due to the challenge of isolating enriched populations from surrounding anther tissues [50]. The expression profile of *Arabidopsis* meiocytes indicates a partial reactivation of silenced transposable elements (TE) associated to *Copia*-like and *Gypsy*-like long terminal repeat (*LTR*) elements with a preferential enrichment of one of the non-*LTR* retrotransposon families, the short interspersed nuclear elements (*SINE*) [51]. TE activation in meiocytes was correlated with co-expression of neighbouring genes and with localized changes in chromatin structure, thought to facilitate meiotic progression [51]. Transient TE element activation in the last diploid stage before meiosis could thus represent an opportunity to introduce genomic variability or to

generate siRNAs that, if not deleterious for meiosis or pollen development, could function in germline specification and/or be transmitted to the next generation.

The differentiation of the male gametophyte (post-PMI) involves chromatin modifications and changes in methylation patterns that lead similarly to the female gametophytes (see below) to the production of two epigenetic dimorphic cell types. During pollen development, the ubiquitous centromeric histone *HTR12* is first detected in unicellular microspores (UNM), after PMI and PMII *HTR12* remain expressed in the generative nucleus and sperm cells, respectively, but is not detected in the vegetative nucleus. Moreover, upon PMII, the vegetative nucleus and sperm cells present distinct histone-based signatures with *HTR5*, *HTR8* and *HTR14* detected in the vegetative nucleus, while sperm cells express *HTR5* and the sperm-cell specific H3.3 variant *HTR10* (also known as *MALE GAMETE SPECIFIC HISTONE H3*, *MGH3*). Thus, sperm cell chromatin becomes distinct from the non-gametic lineage (vegetative nucleus) during pollen development [52, 53].

Significant advances in fluorescence-activated cell sorting (FACS) of *Arabidopsis thaliana* pollen, of its individual cellular components (vegetative nucleus and sperm cells) and of its progenitor cell, the unicellular microspore, were the prerequisite to decipher transcriptomic and epigenetic changes occurring during pollen development. In a first approach, sorted male gametes expressing GFP under a sperm cell specific promoter were used to characterize their transcriptome (mRNA and small RNAs) and the methylation pattern of specific transposons [35, 54, 55]. In order to analyse epigenetic changes during pollen development on a whole-genome scale, this method was further improved to allow simultaneous sorting of the vegetative nucleus and sperm cells as well as FACS isolation of microspores [56, 57]. In a similar fashion, but using DNA dyes instead of fluorescent protein labels, the epigenome of the male germ unit of wild type and mutant pollen grains was analysed [58–60]. Together, these efforts led to genome-wide maps of cytosine methylation in all three sequence contexts (symmetric CG, CHG and asymmetric CHH methylation, where H stands for any residue except G) for sorted microspores, vegetative nuclei and sperm cells. The in-depth analysis of these data contributed significantly to our current understanding of epigenetic reprogramming in the male gametophyte [57, 58] (Fig. 6.3). Through mapping of differentially methylated regions (DMRs) in their genomic context (genic, intergenic or transposable elements) and combination with small RNA abundance, it became apparent that the large majority of these DMRs affect transposon and repetitive element sequences. Their reactivation leads to the production of siRNAs capable of controlling their activity either by transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS). A relaxation of control over potentially harmful transposable elements, particularly in the gametophyte harbouring the germline, seems contradictory. However, the emerging picture is more that of an epigenetic pathway that has been co-opted for inheritance of epigenetic marks (epialleles and imprinted genes) and control of transposable elements during gametophyte development and embryogenesis. The first indication for epigenetic activation of transposable elements in the male gametophyte of *Arabidopsis* came from a study by Slotkin et al. [35]. These retrotransposons get activated in the vegetative nucleus but not in the sperm

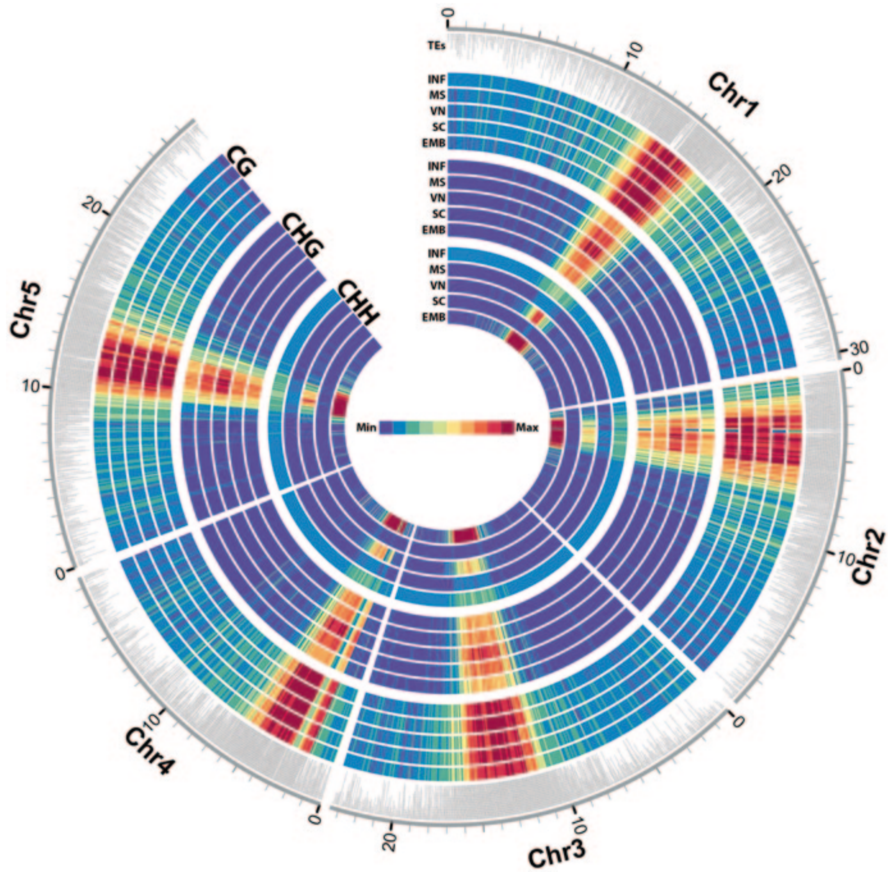

Fig. 6.3 Whole-genome representation of DNA methylation dynamics during pollen development. The heat map is based on bisulphite sequencing data of genomic DNA extracted from inflorescence and embryo as well as distinct pollen cell types and nuclei. Methylation density is shown in 10 kb blocks in the three sequence contexts (CG, CHG and CHH) and along the five chromosomes (low in *blue*; high in *red*). The outer track in grey represents transposable element density. Loss of CHH methylation in microspore and sperm cells in comparison with the vegetative nucleus is evident in the pericentromeric regions. INF inflorescence, MS microspore, VN vegetative nucleus, SC sperm cell, EMB embryo. (Adapted from [57] with permission from Elsevier)

cells, coinciding with differential expression of the chromatin-remodeler *DDM1*, whose function is crucial to maintain DNA methylation of transposable elements. It does so by facilitating access of DNA methyltransferases to linker histone H1-containing heterochromatin [61] and complete loss of *DDM1* leads to increasing developmental abnormalities in inbred lines caused by TE activation [11, 62]. First indicated by transcriptional profiling of *Arabidopsis* sperm cells [54] it was confirmed by translational fusion with GFP that expression of *DDM1* in the male germ unit is restricted to the sperm cells [35]. The transcriptional activation of *Athila* retrotransposons in the vegetative nucleus leads to the production of 21-nt siRNAs in

pollen, which surprisingly accumulate to higher levels in the sperm cells than in the vegetative nucleus [35]. In the sperm, they are thought to reinforce retrotransposon silencing post-transcriptionally (Fig. 6.1a), but they could also play a role during embryogenesis, if delivered upon fertilization (discussed below).

Interestingly, the transcriptional activation of *Athila6A* family retrotransposons in the VN also leads to the production of *Athila6A*-derived 21-nt siRNA854. In pollen, this siRNA acts *in trans* via incorporation into AGO1 and results in post-transcriptional cleavage of *UBP1b* mRNA [63]. In the sporophyte, it has been shown that UBP1b protein is localized to stress granules and is involved in repressing Athila6 GAG capsid protein production, if the transposable element is transcriptionally activated upon stress conditions and the RNAi pathway is non-functional. Here, siRNA854 acts as a suppressor of host transposable element silencing via translational inhibition of UBP1b [64].

The most obvious differences in methylation profiles between VN, SC and microspores were observed for asymmetric CHH methylation in the pericentromeric regions, with the microspore and SCs showing hypomethylation in comparison to the VN [57, 58] (Figs. 6.1a and 6.3). These CHH DMRs map mostly to class I *LTR/Gypsy* retrotransposons and the sperm cells contain 21-nt siRNAs matching the hypomethylated retrotransposons. These siRNAs are likely to be involved in PTGS, thus preventing hazardous transposition of these elements in the male germline. The observed loss of CHH methylation in the male gametes, but not in the VN, is best explained by a passive loss of CHH methylation during microgametogenesis due to reduced maintenance of CHH methylation in the germline. Supporting this hypothesis, expression of the important RdDM component *DOMAINS REARRANGED METHYLTRANSFERASE 2* (*DRM2*) is restricted to the VN during pollen development, with very low levels in the microspore, generative cell and sperm cells [57] (Fig. 6.1a).

But the vegetative nucleus also shows hypomethylated regions, although in a CG sequence context and to a much lesser extent [57, 58]. CG and CHG symmetric methylation is maintained during cell divisions by DDM1 and the DNA methyltransferases MET1 and CHROMOMETHYLASE 3 (CMT3). *MET1* and *CMT3* are expressed during pollen mitosis I and II [65] and levels of symmetric methylation levels in the vegetative cell and sperm cells are largely unaltered when compared to those in somatic cells. Therefore, the observed partial loss of CG methylation in the VN must be achieved through active de-methylation by the DNA GLYCOSYLASE DEMETER (DME) and/or its homologous REPRESSOR OF SILENCING 1 (ROS1), DEMETER-LIKE 2 (DML2) and DML3 [59]. And indeed, *DME* and *ROS1/DML2/DML3* (*RDD*) are not expressed in the sperm cells, but they are expressed in the vegetative nucleus and lead to CG hypomethylation of class II DNA transposons of the *MuDR* and *RC/Helitron* class [57, 59]. It has been shown that imprinted genes are often neighboured by such DNA transposons [66, 67]. In fact, a number of TEs flanking imprinted genes lose CG methylation in the vegetative nucleus relative to microspores and sperm cells. But mainly those flanking genes that are maternally expressed in the endosperm (MEGs) and not those close to paternally expressed genes (PEGs) show high levels of CHH methylation in microspores and

sperm cells. This observed preservation of CHH methylation at TEs neighbouring MEGs depends at least partially on the activity of DME at the corresponding loci in the vegetative nucleus, as indicated by *dme*/+ sperm cells showing reduced CHH methylation when compared with wild type sperm cells [58]. In addition, 24-nt siRNAs corresponding to MEGs accumulate preferentially in the sperm cells [57], supposedly promoting MEG-specific CHH methylation (Fig. 6.1a).

Methylation of recently discovered hypervariable epialleles might be affected in a similar way. Through analysis of leaf methylation profiles after 30 generations of inbreeding by single-seed descent, it could be shown that more than 100 loci are prone to gain methylation sporadically and recurrently [68, 69], many of those being targets of RDD. More than half of the 100 loci are hypermethylated in sperm cells, readily explained by the low levels of *DME/RDD* expression in microspores and high expression in the vegetative nucleus [57]. This hypermethylation in sperm cells supports the idea that hypervariable epialleles already methylated in the inflorescence might be heritable through appropriate epigenetic reprogramming in the male germline [68, 69].

As outlined above, epigenetic reprogramming during male gametogenesis in *Arabidopsis* results in two cell types with distinct epigenetic features (Fig. 6.1a). Most notably, the vegetative nucleus undergoes extensive reprogramming involving loss of pericentromeric heterochromatin, loss of CG methylation and production of 21-nt siRNAs from activated retrotransposons. In this context, it is important to remember that the vegetative nucleus does not contribute genetic material to the next generation. In relation to the sperm cells the vegetative nucleus functions as a nurse cell, similar in that sense to the role the central cell plays for the egg cell (see below). Thus, partly due to the reprogramming of its companion cell, but also due to alterations in its own epigenetic make-up, the male gametes represent a unique epigenetic state upon anthesis: (A) *LTR/Gypsy* retrotransposons are hypomethylated in a CHH context, (B) 24-nt siRNAs matching TEs that flank imprinted, maternally expressed genes are abundant, (C) 21-nt siRNAs matching VN-activated retrotransposons accumulate.

Exactly how and when this sequestration of 21- and 24-nt siRNAs into the male gametes is occurring is still a matter of debate. Theoretically, these siRNAs could move from the vegetative nucleus through the cytoplasmic extension of the male germ unit or via the pollen cytoplasm. And indeed, an artificial microRNA (*amiR*) expressed in the vegetative nucleus that targets a sperm cell expressed mRNA encoding GFP led to a reduction in GFP signal [35]. It has been argued however that the *LAT52* promoter used to drive expression in the vegetative nucleus is already active at the microspore stage and therefore the *amiR* could have been carried over to the precursor of the sperm cells, the generative cell, already during pollen mitosis I. Support for this hypothesis comes from a study using a promoter specific to the vegetative cell (post PMI, late bicelullar pollen) to drive *amiR*GFP expression, in which case germline-specific GFP expression could not be silenced [70]. Regardless of the origin of siRNAs accumulating in sperm cells, their role could be to reinforce silencing of maternally expressed genes as well as a subset of retrotransposons in the male gamete. An alternative function however could only come to bare after their delivery to the egg cell and during early embryogenesis (Fig. 6.1c).

Epigenetic Regulation in the Female Gametophyte

The specification of MMC from surrounding somatic cells in ovule primordia seems to be accompanied by extensive chromatin reprogramming important to establish an epigenetic and transcriptional landscape distinct from the surrounding somatic tissues. Chromatin decondensation associated with depletion of linker histones, reprogramming of histone variants and establishment of histone marks characteristic of a more permissive chromatin state, contribute to an epigenetic landscape supporting pluripotency and competence for a postmeiotic development of the female gametophyte [71].

The specification of a single functional MMC in a nucellus primordium seems to be epigenetically regulated through the action of small non-coding RNAs [48, 72]. Maize *AGO104,* closely related to *Arabidopsis AGO9,* is required for meiosis II during megasporogenesis [73]. Maize *ago104* mutants develop normal MMCs, but fail to undergo meiosis and instead undergo a mitotic-like division followed by megagametogenesis to produce functional unreduced megaspores [73]. In *Arabidopsis ago9* mutants, the somatic cells adjacent to MMCs acquire a reproductive cell fate bypassing meiosis and generating multiple unreduced megaspores [72, 74]. Thus *AGO9* seems to act by repressing germ cell fate in somatic tissues, while *AGO104* acts to repress somatic fate in reproductive cells. These processes most resemble diplosporic and aposporic development in apomictic plants, suggesting that the regulation of these two gametophytic pathways could be interconnected [72, 73]. AGO5 and AGO9 are known to preferentially associate with 24-nt small interfering RNAs (siRNAs) derived from transposons and repetitive elements but with different specificities to the 5′-terminal nucleotide. The specific accumulation of AGO104 and AGO9 in somatic nucellar cells, but their absence from MMCs raised the hypothesis whereby mobile siRNAs derived from transposable elements could move from adjacent somatic cells to MMC to restrict reproductive development to the functional megaspore [73]. *MEL1*, the orthologue of *Arabidopsis AGO5,* is initially expressed in the sub-epidermal cells in the ovule primordia during MMC differentiation, but later the expression becomes restricted to the MMC, disappearing during meiosis [48]. In rice *mel1/ago5* mutants the specification of the reproductive cell lineage is not affected, but MMCs fail to undergo meiosis leading to the absence or arrest of female gametophyte development. In *Arabidopsis, ago5* mutants do not show any visible phenotype, but a semidominant form of AGO5, *ago5-4,* presents defects in the initiation of megagametogenesis [49]. The *ago5-4* truncated form lacking the MID domain and catalytic PIWI domain may compromise sRNA binding efficiency, reminiscent of viral suppressor proteins which sequester siRNAS [75]. Consistently, the expression of the viral RNAi suppressor of 24-nt siRNAs, *P1/Hc-Pro*, in somatic nucellus cells produced a similar phenotype, supporting that RNA-directed DNA methylation pathways act in somatic nucellar cells to initiate megagametogenesis [49]. While a possible redundancy of *AGO5* cannot be excluded, these studies support the existence of two RdDM independent pathways, one acting through *AGO9* restricting the reproductive potential to the functional megaspore [72, 73] and an independent pathway initiating megagame-

togenesis [49]. Also supporting this hypothesis, the loss of function *of dmt102* and *dmt103* in maize, homologous to *Arabidopsis CMT3* and *DRM2,* produces unreduced gametes and ectopic embryo sac formation from supernumerary MMCs [76], suggesting that RNA-directed DNA methylation pathways are associated to transcriptional repressive states that could determine the distinction between an apomictic and a sexual reproductive development [76]. Interestingly, *AGO5* and *AGO9* also show enriched expression in the sperm cells [54, 55], but no specific function has been identified in the male gametes so far.

In contrast to male gametogenesis where two identical sperm cells are formed with the same potential to fertilize any of the female gametes [39], female gametogenesis gives rise to two dimorphic female gametes, the egg and the central cell, which contribute to distinct fates in post-fertilization products, the embryo and the endosperm (Fig. 6.1b, c). As all female gametophytic cells are genetically identical, it was proposed that female gamete dimorphism could reflect individual epigenetic states and these should be established in the syncytium stage, prior to embryo sac cellularization and differentiation [77]. Epigenetic reprogramming of female gametes by DNA methylation, histone modifications and replacement of histone variants contribute to post-fertilization dimorphic epigenetic states regulating transposon activity with functional implications in acquisition of zygotic totipotency and initiation of embryogenesis [78–80]. In particular, the composition and incorporation of specific histone variants contributes to establish specific chromatin epigenetic states in the egg and central cell [77]. The central cell expresses several *H3.1* isoforms and shows enrichment for two specific *H3.3* variants (*HTR8* and the unusual *HTR14*), while the mature egg cell is depleted of *H3.1* variants, expressing a single *H3.3* variant (*HTR5*) [52]. Moreover, egg cells exhibit a hypermethylated quiescent status correlating with low levels of transcriptional activity of PolII and enrichment of repressive chromatin histone marks associated predominantly with silenced states in euchromatic and heterochromatic regions (*H3K9me2* and *LHP1*). In contrast, the central cell is hypomethylated and transcriptionally active, correlating with a more permissive chromatin configuration. The dimorphic *H3K9me2* chromatin marks seem to be regulated by CMT3 in the egg cell and by DEMETER-LIKE (DML) activity in the central cell [77, 81]. Consistently, female gametes present an asymmetry of DNA methylation patterns, largely associated to the reduced activity of DME in the central cell [82, 83] and transcriptional repression of DNA methyltransferases like *MET1*, mediated by the retinoblastoma pathway [84, 85]. Passive loss of DNA methylation in the egg cell appears to be counteracted by *de novo* DNA methyltransferases DRM1 and DRM2 while in the central cell only low levels of *DRM2* were detected [86]. The requirement of CMT3-induced egg cell silencing in both transposon and euchromatic regions led to the hypothesis that siRNAs produced in transcriptional active cells (such as central cell or somatic cells) could target the egg cell [81]. DRM2 activity relies on RNA polymerases PolIV and PolV that have evolved an exclusive function in siRNA biogenesis and transcriptional silencing, respectively [87]. PolIV-dependent 24-nt siRNAs*,* many of which generated from TEs*,* were shown to be maternally specific and accumulate predominantly in the central cell [58, 88]. The finding that AGO9 associates with

24-nt siRNAs derived from transposable elements and is expressed in somatic ovule cells but not in female gametes raised the possibility that somatic cell-derived siRNAs could act as a non-cell-autonomous signal travelling to female gametophytic cells to restrict TE activity. This hypothesis was also consistent with reactivation of TEs in the egg and synergid cells of *ago9* mutants [72, 74].

Post-fertilization Epigenetic Reprogramming

In two parallel events, sperm cells fertilize the egg cell and the central cell and after karyogamy the distinct cell fate of both fertilization products, the zygote and the endosperm, is reflected in distinct epigenetic profiles (Fig. 6.1c). In the endosperm, which nurtures embryogenesis during seed development [89], low levels of DNA methylation are achieved through active DME-driven demethylation [83, 90] and continued repression of the main methyltransferases *MET1, CMT3* and *DRM2* [82, 86, 91] set a profile that most resembles the epigenetic make-up of the transcriptionally active central cell before fertilization. In the embryo, a crucial epigenetic reprogramming reinforces maintenance of DNA methylation in CG and CHG contexts by *MET1* and *CMT3* expression and RdDM activity with high expression levels of *DRM2* assures the gradual *de novo* methylation in CHH contexts (Fig. 6.4), which may contribute to maintain stable inheritance of epialleles across generations, reset silenced imprinted genes in the embryo or reinforce TE silencing contributing to genomic stability.

In the embryo methylation of the hypomethylated *LTR/Gypsy* retrotransposons coming in from the paternal side is restored, possibly through CMT2-dependent re-methylation pathways [61], although 21-nt siRNA might also be involved at the post-transcriptional level. These siRNAs could have their origin in the central cell or endosperm. That such movement of siRNAs from the female companion cell (central cell) to the egg cell is possible was shown in an experiment analogous to that in pollen, in which an artificial miRNA expressed in the central cell led to the reduction of a GFP signal in the egg cell [58].

In *Arabidopsis,* delivery of paternal 21-nt siRNAs targeting maternal *LTR* retrotransposons post-transcriptionally and simultaneous transcriptional targeting of CHH hypomethylated paternal retrotransposons by 24-nt siRNA of maternal origin could potentially create a mix and match situation of hypomethylated TEs and their targeting siRNAs upon fertilization (Fig. 6.1c). Such interaction between the 21-nt post-transcriptional and 24-nt transcriptional pathways has been predicted [87] and if occurring during reproduction it could have far-reaching consequences for the embryo and endosperm [92]. Two scenarios are conceivable: (A) Transposon sequences of the two genomes involved in an interspecific cross differ to an extent that the siRNA sequences derived from one parent do not match the transposable elements in the other parent. (B) Interploidy crosses lead to a genomic dosage disequilibrium characterized by the siRNA pool of one parent being insufficient to silence all copies of transposable elements of the other parent. These mechanisms

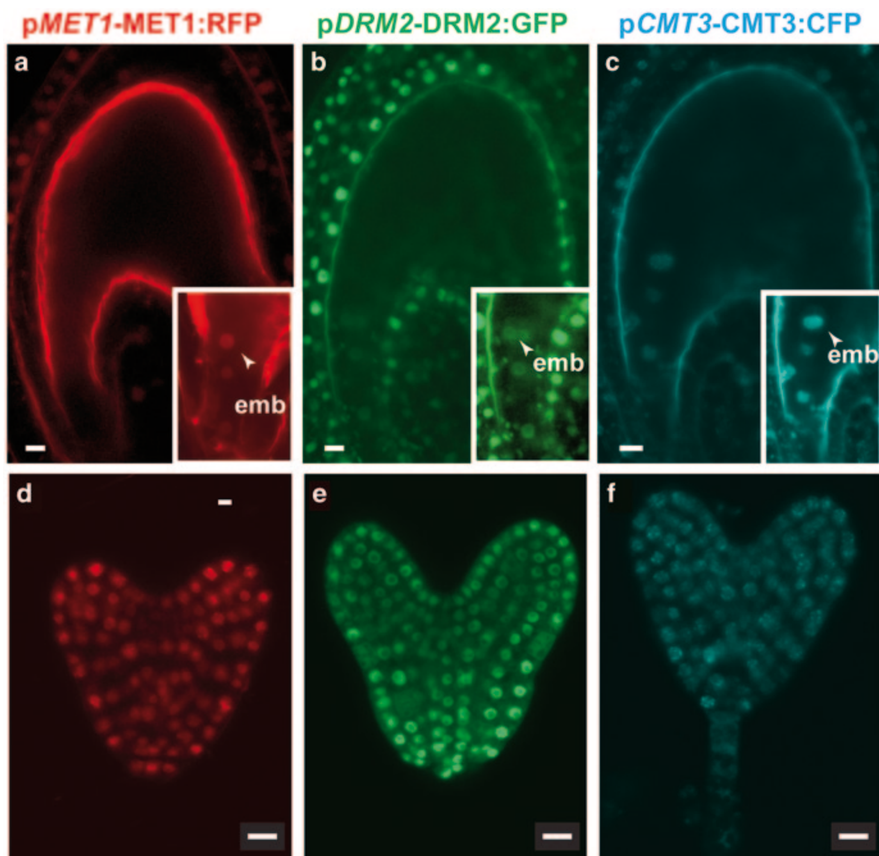

Fig. 6.4 Expression pattern of DNA methyltransferases during seed development. During seed development in *Arabidopsis,* the DNA methyltransferases *MET1*, *CMT3* and *DRM2* are differentially expressed in the developing embryo and its nourishing endosperm, as visualized using fluorescent fusion proteins under the control of the respective native promoter. During early embryogenesis (A–C), expression of the DNA methyltransferases is detected in the nuclei of the one cell (*MET1*) and two cell embryo (*CMT3* and *DRM2*), respectively (arrowheads in inset). The endosperm does not show any detectable expression levels. This divergent expression pattern is maintained during embryogenesis as exemplified by exclusive expression in the embryo at the heart stage (D–F). Scale bar: 20 µm. Thus, DNA methylation in all sequence contexts is highly active during embryogenesis, while it is strongly reduced in the endosperm. (Adapted from [86] with permission from Elsevier)

may present an effective way to assess parental compatibility at fertilization, in case of hybrid incompatibility leading to failure to suppress transposon activity (and deregulation of siRNA/TE controlled genes in their vicinity), ultimately resulting in seed abortion [92–94]. Such seed abortion has been observed for instance in interspecific crosses between *Arabidopsis thaliana* and *Arabidopsis arenosa,* involving upregulation of a normally silenced paternal *ATHILA* retrotransposon in a genome dosage-sensitive way [95, 96]. Underlining a general role of small RNAs

for hybridization barriers, hybrid dysgenesis in *Drosophila melanogaster* involves the reactivation of paternally inherited transposable elements in the progeny, caused by the lack of maternal Piwi-interacting RNAs targeting these TEs [97].

Delivery of small RNAs by the male gametes to the egg cell or central cell has not been proven in plants, but further support for such hypothesis comes from a delivered mRNA. *Short Suspensor* (*SSP*) mRNA accumulates in sperm cells of mature pollen and is delivered to the egg cell at fertilization, where its translation in the zygote triggers the activation of the YODA MITOGEN-ACTIVATED PROTEIN MAP kinase cascade responsible for the regulation of the first asymmetric division of the *Arabidopsis* zygote [98]. Similar to plants, human sperms contain a complex repertoire of coding and non-coding RNAs, but the role the delivery of this paternal RNA pool might play upon fertilization remains to be shown [99, 100]. In contrast, the picture is much clearer in *C. elegans*. Here, compelling evidence indicates that sperm carries ARGONAUTE/small RNA complexes transmitting a transgenerational small RNA memory of paternal gene expression [101].

The selective erasure and maintenance of epigenetic modifications at fertilization may also be critical for zygotic genomic activation (ZGA) which marks the initiation of *de novo* transcription from the zygotic genome. ZGA is essential for the transition from a quiescent transcriptional stage to a pluripotent state in early embryo enabling them to activate any pathway required for organism development [102]. In *Arabidopsis*, maternal transcripts predominate in a 2–4 cell embryonic stage, showing a gradual transition to increased paternal contribution at the globular stage [103]. Consistent with these observations *Arabidopsis* embryo development proceeds up to a globular stage even with low levels of active PolII, suggesting that early stages of embryo development rely on stored maternal transcripts [104, 105]. Specific maternal epigenetic marks were associated with the initial quiescent zygotic stage and with zygotic activation of paternal alleles which should result from a balance between chromatin-based repressive mechanisms and the establishment of a permissive chromatin state for transcriptional activation [103]. Consistently, in *Arabidopsis*, H3.3 variants from both gametes are quickly removed from the zygote after karyogamy, and somatic H3 variants are restored by *de novo* synthesis in the embryo, while in the endosperm there is a progressive dilution of the H3.3 variants through successive nuclear divisions [52, 53]. This epigenetic reprogramming in the egg cell seems to be mediated by CAF1 [103], ultimately limiting the inheritance of epigenetic information carried by H3.3 variants to the next generation [52]. In a process not well understood, H3K9 methylation driven by the maternal allele of *KRYPTONITE* (*KYP*) and *CMT3* as well as siRNAs produced by RdDM pathways that predominantly target TEs [106] seem to target also coding genes, which is believed to set the stage for zygotic activation and embryo development [103].

Genomic imprinting in plants occurs after fertilization whereby specific loci in endosperm are expressed during seed development according with their parental origin. Most imprinted genes are associated with differentially methylated regions (DMRs) that are methylated in a CG context in one of the two parental alleles. This CG methylation is important for epigenetic inheritance through gametogenesis and involved with imprinting in the endosperm [84, 107–109]. Several examples

of genomic imprinting in plants highlight the role of two distinct and interdependent mechanisms, DNA methylation and polycomb group (PcG) complex-mediated H3K27me3, in setting parental-specific epigenetic marks that are established in gametes and erased post-fertilization in endosperm and embryo. *FWA* and *MEDEA* maternal imprinting in endosperm are a good example of this epigenetic regulatory control: during fertilization, DME removes CG methylation in the *FWA* maternal allele, sustaining the maternal monoallellic expression in endosperm while the low activity of *MET1* in the endosperm is sufficient to maintain CG methylation of the *FWA* paternal allele [82, 86, 91]. On the other hand, *MEDEA* uses a more complex mechanism in which DME in the central cell demethylates CG sites flanking *MEDEA*, promoting *MEDEA* maternal expression in endosperm. MEDEA then binds to the Fertilization Independent Seed (FIS)-PcG complex. After fertilization in the endosperm the PcG complex represses the expression of the paternal allele through enrichment of repressive histone marks (H3K27me3). Moreover, the mechanism by which *PHERES1 (PHE1)* is paternally imprinted in endosperm involves a DMR in the 3′ end of *PHE1* and recruitment of PcG complex. In the central cell, the DMR in *PHE1* 3′ end is hypomethylated, allowing recruitment of FIS-PcG complex to *PHE1* promoter and resulting in stable maternal *PHE1* repression in the endosperm. The FIS complex is absent in sperm cells and the methylated DMR prevents the silencing activity, causing the paternal allele to be active in the endosperm ([110] and references therein).

Until recently, few genes were known to be imprinted in the embryo and for this reason the regulatory control was poorly understood [111]. The study of *MATERNALLY EXPRESSED IN EMBRYO 1* (*MEE1*) gene of maize, imprinted in both the embryo and endosperm, has shown that imprinted alleles can acquire DNA methylation after fertilization. The demethylated state of maternal *MEE1* in the central cell and the methylated paternal allele are consistent with the exclusive maternal expression of *MEE1* in endosperm. In the egg cell, the maternal *MEE1* is methylated but upon fertilization is rapidly de-methylated, establishing differential parental epigenetic states in the embryo. During embryo development the maternal allele is then remethylated resetting the maternal imprinting marks later in embryogenesis [111]. This embryonic *MEE1* maternal imprinting is consistent with an active demethy-lation in the zygote; however, the mechanism of regulation raised some intriguing questions implying the existence of an allele-specific recognition mechanism that could distinguish between two equal methylated parental alleles [78, 111]. One possible hypothesis is that 24-nt siRNAs accumulating in the endosperm could be transported to the embryo [58, 90] to guide demetylation of the maternal *MEE1* allele in the embryo [112]. Evidences show that siRNAs interacting with ROS3 are sufficient to guide sequence-specific demethylation involving ROS1 [113], supporting the possibility that siRNAs can also guide DNA demethylation. However, it still remains to be shown that strand-specific differences exist between parental alleles. Such possibility could be achieved by differential histone marks in parental alleles. The recent identification of several genes imprinted in embryo do not exclude this hypothesis and support that DNA methylation is not a primary imprinting mark in embryos. The regulation of embryo imprinting seems to be partially imposed by H3K27me3 mediated by the PRC2 complex but independent of MET1

[114]. While genomic imprinting in the endosperm does not raise major implications for the following generation, evidences indicate that imprinting in the embryo is maintained during embryogenesis and early seedling development, but PEG and MEG imprinting marks need to be eventually erased and reset before reproductive development in the next generation [114].

The fact that a subset of maternal and paternal imprinted expressed genes are associated with DMRs, probably deriving from accidental silencing of repetitive elements neighbouring these genes, led to the hypothesis that genomic imprinting could have evolved as a by-product of TE silencing [115]. Recent evidences support that RdDM pathways acting in plant gametes can regulate parental-specific genomic imprinting at specific loci in endosperm [116]. In early endosperm development, low RdDM activity together with low activity of *MET1* and other methyltransferases [86, 91, 117] is expected to contribute to maintenance of the demethylated state of the maternal allele and activation of the maternal imprinted genes (MEGs). Silencing of the paternal alleles seems however to be dependent on RdDM pathways, whereby NRPD2A-dependent siRNAs generated potentially from activity of transposon elements in diploid somatic paternal tissues seem to be sufficient to target *de novo* DNA methylation and silencing prior to gametogenesis. The methylated status of paternal alleles is maintained by MET1 during sporophytic pollen development, sustained in the haploid gametes and inherited after fertilization in endosperm [116].

The observation that most imprinted genes in plants and animals are expressed in the placenta and endosperm, respectively, tissues allocated to nutrition of the developing embryo gave rise to the prediction that the function of imprinted genes in endosperm could have played a major role in the evolution and selection of genomic imprinting. The parental conflict theory predicts that nutrient allocation in the offspring is resolved in favour of the parents reproductive interests, i.e. maternally expressed imprinted genes should negatively regulate endosperm growth, while paternal imprinted genes positively regulate endosperm growth [118]. While this is the case for many of the identified imprinted genes, the *MATERNALLY EXPRESSED GENE 1* (*MEG1*) in maize has opposite effects promoting seed growth [119]. In addition, the lack of obvious functions and the fact that ectopic expression of many of these imprinted genes in vegetative tissues do not cause deleterious effects in plant development [120, 121], have led to a new hypothesis in which, genomic imprinting might have evolved from positive selection of beneficial mutations in vegetative tissues that were then retained and co-opted for positive functions in embryo or endosperm [120].

The epigenetic reprogramming observed in the gametes and their products has implications that go beyond their alleged roles for transposon and imprinting control during the reproductive phase. This could be the case for the observed variability of epialleles across generations [68, 69], possibly driven by gains and losses in CHH methylation during gametogenesis and early embryo development [122]. In this respect it should also be noted that both 21- and 24-nt siRNAs can be transported over long distances between shoot and root (and *vice versa*) and that these mobile 24-nt siRNAs are able to direct RdDM in meristematic root stem cells [123–125]. Assum-

ing that transport could also be effective to the shoot meristem and eventually to the gametes, these siRNAs could act in a pathway underlying epigenetic inheritance. This could explain transgenerational memory of stress as observed in plants [126]. Support for this hypothesis comes from salicylic acid stress in the sporophyte leading to the production of TE-associated 21-nt siRNAs that are similar to those that are epigenetically activated in the vegetative nucleus of pollen [127].

Perspectives

In recent years, we have seen significant advances in experimental methods allowing the isolation of specific reproductive cell types or cell-type components. Through combination with genome-scale approaches like bisulphite sequencing and RNAseq, these have led to a better comprehension of the epigenetic landscape associated to regulation of a number of developmental transitions, namely how plants determine a sporogenous reproductive cell lineage from somatic cells, how dimorphic epigenetic states may contribute to male germline differentiation in a multicellular gametophyte or how dimorphic epigenetic states in female gametes contribute to establish distinct developmental programs post-fertilization. However, the epigenetic profiles of several specific developmental transitions are still incomplete due to the challenge of isolating particular cell types (e.g. meiocytes or female gametophytic cells) from their neighbouring somatic cells or in specific developmental stages (e.g. during pollen tube growth), information that will be crucial to improve resolution of epigenetic states in specific cell types and define the precise timing of epigenetic reprogramming during gametogenesis. These reprogramming events are also a prerequisite to establish temporary transcriptional repressive states during cell fate transitions in order to restore totipotency and allow cell-type differentiation programmes.

Moreover, it will be of fundamental importance to understand the regulatory mechanisms that specifically operate during these phase transitions, enabling plants to retain, erase or reset specific epigenetic information acquired during the plant life cycle as well as parent specific allelic expression patterns that can contribute to the next generation. Histone modifications and small RNAs seem to be involved in this transgenerational epigenetic inheritance. It will be of particular interest to understand if mobile siRNAs, either from companion cells or inherited, can modify the epigenetic landscape of the zygote or function as critical regulators of gene expression during embryo development. An exciting development has been the association of epigenetic mechanisms with the potential regulation of apomictic development, raising a profound interest in the epigenetic principles that distinguish sexual reproduction from apomixis. Given the emerging impact of epigenetic processes on several aspects of sexual reproduction in angiosperms future insights hold the promise to yield novel experimental tools, ultimately opening new paths to improve crop species.

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