Epigenetic reprogramming in plant sexual reproduction

Tomokazu Kawashima and Frédéric Berger

Abstract | Epigenetic reprogramming consists of global changes in DNA methylation and histone modifications. In mammals, epigenetic reprogramming is primarily associated with sexual reproduction and occurs during both gametogenesis and early embryonic development. Such reprogramming is crucial not only to maintain genomic integrity through silencing transposable elements but also to reset the silenced status of imprinted genes. In plants, observations of stable transgenerational inheritance of epialleles have argued against reprogramming. However, emerging evidence supports that epigenetic reprogramming indeed occurs during sexual reproduction in plants and that it has a major role in maintaining genome integrity and a potential contribution to epiallelic variation.

Epigenetic marks
Modifications of the chromatin that are inherited through cell division.

Spores
Haploid cells that are derived from meiosis of meiocytes and that undergo several rounds of mitosis to give rise to gametophytes. The male and female spores are also known as microspores and megaspores, respectively.

Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604 Singapore; and Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543 Singapore. Present address: Gregor Mendel Institute of Molecular Plant Biology GmbH, Dr. Bohr-Gasse 3, 1030 Vienna, Austria. Correspondence to F.B. e-mail: frederic.berger@gmi.oeaw.ac.at
doi:10.1038/nrg3685
Published online 22 July 2014

Genome activity in many eukaryotes is modulated by histone modifications and DNA methylation. DNA methylation of cytosine residues in a CG context and the histone H3 lysine 9 trimethylation (H3K9me3) mark are typically inherited through cell divisions in a semi-conservative manner[1–3], although we do not discount that other chromatin modifications (including H3K27me3) may also qualify as epigenetic marks[4,5]. DNA methylation has been the major focus for understanding changes to the epigenome during development and between generations; this focus is reflected here, although we also discuss mechanisms that could be involved in reprogramming histone modifications.

Global changes to the epigenome involve a widespread resetting of epigenetic marks between generations, which we refer to as epigenetic reprogramming. Although comparable to some extent, there are various differences between epigenetic reprogramming in plants and mammals, which is probably a consequence of the differences in reproductive biology (FIG. 1). In mammals, the germ line is defined at an early stage of embryogenesis[6,7] before meiosis. By contrast, meiosis takes place before germline differentiation in plants. Meiotic precursors are determined from differentiated adult somatic cells in a position-dependent manner[8] and undergo meiosis to give rise to haploid spores from which germ cells differentiate[9,10]. These haploid spores are subjected to several rounds of mitosis, which leads to the development of the multicellular female and male gametophytes[11,12] (FIG. 1). During the haploid gametophytic life of flowering plants, a short-lived germ line is established[8]. Fertilization gives rise to the diploid zygote and initiates a new diploid sporophytic phase with the development of an embryo[13]. Moreover, flowering plants are characterized by a second fertilization event that gives rise to an embryo-nurturing tissue known as the endosperm[14] (FIG. 1). The germline specification after meiosis in plants provides a unique multicellular haploid (that is, gametophytic) life phase, and no equivalent life phase transitions exist in animals[8,15].

In mammals, reprogramming of DNA methylation occurs twice in each life cycle. This reprogramming comprises the erasure of DNA methylation marks from the previous generation followed by a re-establishment of DNA methylation. Global demethylation takes place during early embryogenesis in the newly defined germ line and a second time after fertilization in the zygote[6,7]. There is no current consensus regarding the mechanisms that contribute to the erasure of epigenetic marks in mammals[16,17]. The re-establishment depends on *de novo* DNA methyltransferases and associated factors[17–23]. In plants, the persistence of methylated states of some silenced genetic elements (such as transposable elements (TEs), certain genes and transgenes) through generations[21–23] led to the hypothesis that DNA methylation might be inherited in a stable manner from one generation to the next and that epigenetic reprogramming therefore might not exist in plants. However, recent global analyses of the epigenome and detailed molecular studies have shown that epigenetic reprogramming indeed takes place during plant sexual reproduction. Although epigenetic events and the chromatin status remain mostly uncharacterized during meiosis, data indicate an overall reduction of DNA methylation in the germ line during subsequent gametogenesis.

Gametophytes
Haploid life forms that define the germ line and that are produced by the development of spores. Each gametophyte generally comprises a small number of cells, such as the embryo sac (female gametophyte) and the pollen (male gametophyte) in flowering plants. However, in mosses, the gametophyte constitutes the major part of the life cycle.

This is partly due to the activity of demethylases of the DEMETER (DME)/REPRESSOR OF SILENCING 1 (ROS1) family, which have evolved specifically in plants[24,25]. After fertilization, the partially demethylated genome of the zygote seems to undergo dynamic *de novo* DNA methylation during embryogenesis, which is mediated by *de novo* DNA methyltransferases as in mammals.

In this Review, we focus on recent discoveries that highlight dynamic changes of DNA methylation during plant sexual reproduction. We cover both maternal and paternal soma–germ specification, sporogenesis and gametogenesis, as well as subsequent fertilization and embryogenesis. We also discuss the biological implications of these epigenetic reprogramming events. First, to introduce the relevant molecular components, we briefly mention the pathways that are involved in *de novo* and maintenance methylation, but further molecular details of these pathways are reviewed elsewhere[26–28].

DNA methylation mechanisms in plants

In *Arabidopsis thaliana*, the most widely used plant model organism, DNA methylation occurs at cytosine residues in all DNA contexts (CG, CHG and CHH, where H represents A, C or T). Methylation in each context is primarily controlled by distinct DNA methyltransferases[28,29] (BOX 1) and is linked to specific biological functions. DNA methylation in promoters and heterochromatin is associated with H3K9me2, and these epigenetic marks collectively participate in gene silencing, particularly to repress TE activity[29–31].

A large proportion of DNA methylation depends on DNA METHYLTRANSFERASE 1 (MET1)[32], which is recruited at the DNA replication site and replicates patterns of CG methylation in a semi-conservative manner[33] (BOX 1). In contrast to the repressive functions of CG methylation in promoters and heterochromatin, CG methylation in gene bodies is broadly associated

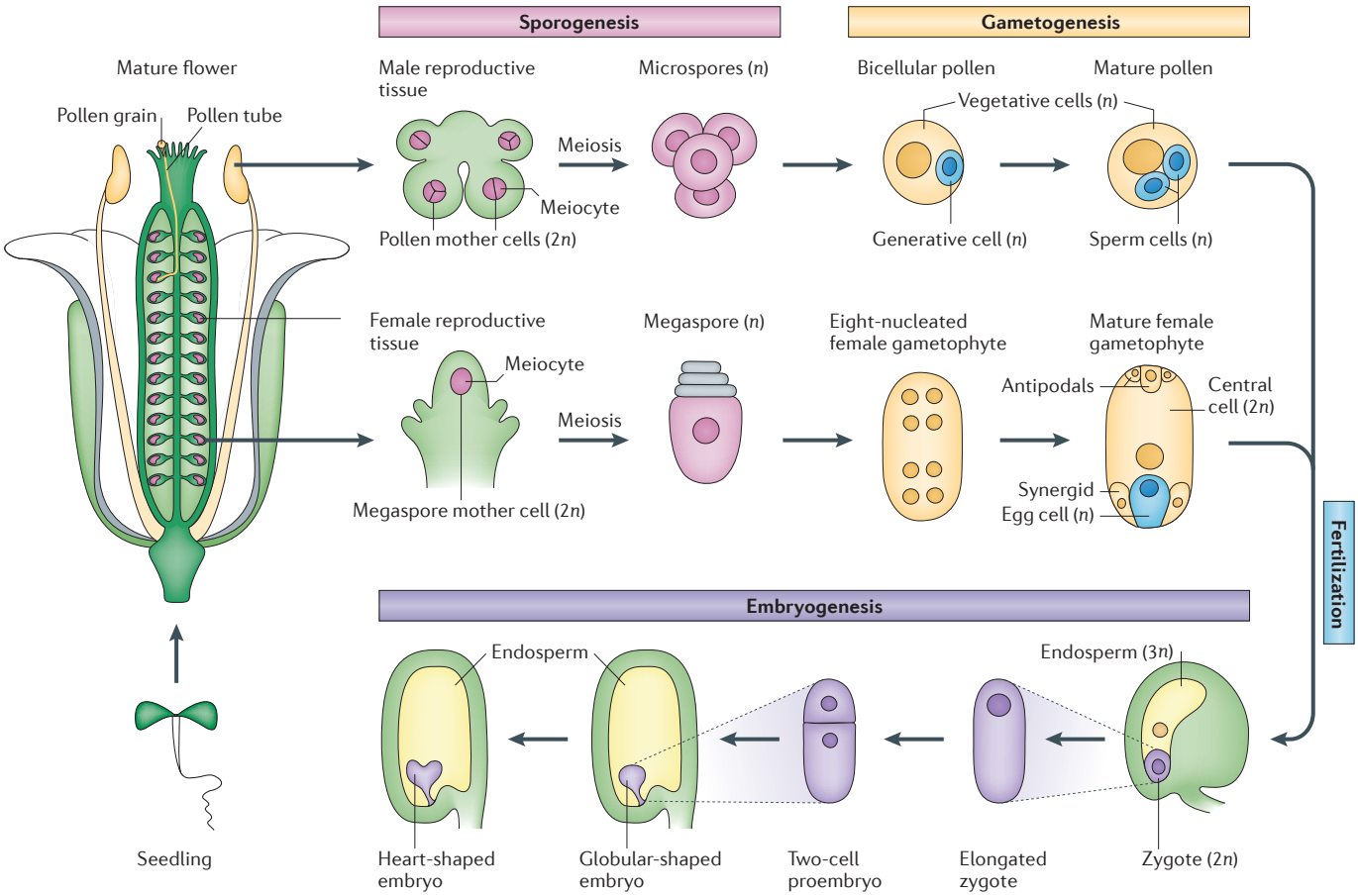


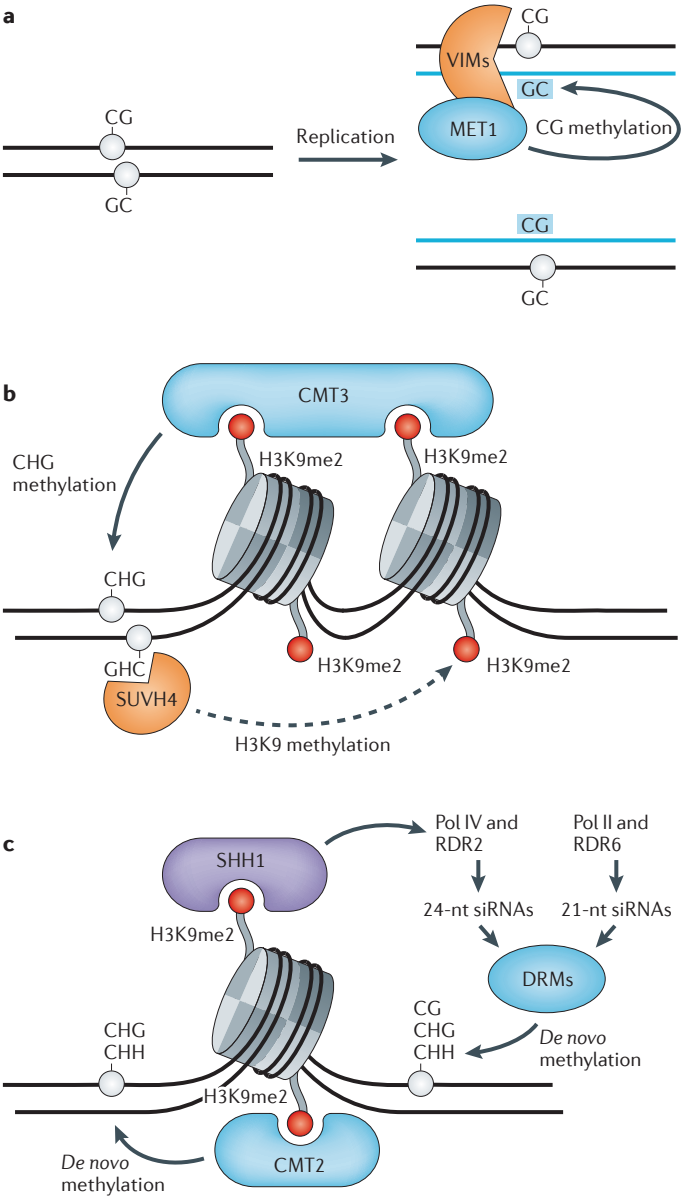
Figure 1 | **Sexual reproduction in *Arabidopsis thaliana*.** In the flower of *Arabidopsis thaliana*, pollen mother cells and megaspore mother cells are generated in a position-dependent manner from somatic cells in the male and female reproductive tissues, respectively. Meiosis takes place and generates microspores from pollen mother cells and megaspores from megaspore mother cells. The microspore undergoes asymmetrical division to give rise to the vegetative cell and the generative cell. The generative cell divides once more to create two sperm cells within the vegetative cell, which leads to the mature pollen. Conversely, the megaspore is subjected to three rounds of nuclear division to generate a syncytial female gametophyte with eight nuclei. Cytokinesis then takes place to establish the mature female gametophyte, which consists of the egg cell, the central cell and accessory cells (antipodals and synergids). *A. thaliana* sperm cells are immotile and are delivered to the female gametophyte by the pollen tube that elongates from the vegetative cell. The egg cell and the central cell are each fertilized by one sperm cell to produce the zygote and the endosperm, respectively. Unlike in animals, the elongation of the zygote precedes the first cell division, which initiates a proembryo that develops into the globular-shaped embryo and then the heart-shaped embryo to become the mature seed.

Box 1 | **Models of CG, CHG and CHH DNA methylation**

Factors involved in the maintenance of DNA methylation in mammals are well characterized, and mutations in *Arabidopsis thaliana* homologues show the same defects in DNA methylation maintenance, which implies that the CG methylation mechanism is similar between mammals and plants[28]. In brief, the hemimethylated CG sites generated during DNA replication are thought to be recognized by *A. thaliana* VARIANT IN METHYLATION (VIM; also known as ORTHRUS) family proteins[116,117]. VIMs then recruit DNA METHYLTRANSFERASE 1 (MET1) to accomplish the fully methylated CG state to maintain CG methylation patterns following DNA replication (see the figure, part **a**).

CHG methylation in *A. thaliana* is maintained by a feedback loop that involves histone H3 lysine 9 dimethylation (H3K9me2)[33] (see the figure, part **b**). *A. thaliana* CHROMOMETHYLASE 3 (CMT3), which is a CHG DNA methyltransferase, binds to H3K9me2 and methylates DNA at nearby CHG sites[37]. The methylated CHG DNA recruits SU(VAR) HOMOLOGUE 4 (SUVH4), which is one of the histone methyltransferases that is important for H3K9 dimethylation[118,119]. SUVH4-mediated deposition of H3K9me2 marks on nucleosomes around the methylated CHG DNA creates a CHG–H3K9me2 positive feedback loop. The crystal structure of the maize CMT3 homologue showed that it can bind to two H3K9me2 peptides[37]. It is still unclear whether CMT3 binds to two H3K9me2 marks from two distinct nucleosomes or within the same nucleosome; however, this dual binding suggests that CMT3 may not only increase its binding affinity towards H3K9me2-enriched loci for their preferential methylation but may also 'walk' along nucleosomes that contain H3K9me2, which is characteristic of a potential mechanism to spread chromatin status[37].

There are several pathways that control *de novo* methylation in *A. thaliana* in all sequence contexts (CG, CHG and CHH; see the figure, part **c**). SAWADEE HOMEODOMAIN HOMOLOGUE 1 (SHH1) binds to H3K9me2 and recruits RNA polymerase IV (Pol IV)[120]. Pol IV and RNA-DEPENDENT RNA POLYMERASE 2 (RDR2), together with other components of the canonical RNA-directed DNA methylation (RdDM) pathway, then generate 24-nucleotide (nt) small interfering RNAs (siRNAs), which are recognized by Argonaute proteins to recruit to DOMAINS REARRANGED METHYLTRANSFERASE 1 (DRM1) and DRM2 for *de novo* DNA methylation[27,28]. Conversely, in the absence of DECREASED DNA METHYLATION 1 (DDM1), a non-canonical RdDM pathway involving Pol II and RDR6 generates 21-nt siRNAs that guide DNA methylation[55]. Recently, CMT2 showed non-CG methylation ability through binding to H3K9me2 (REFS 29,35). Phylogenetic conservation of DNA methyltransferases and associated proteins among flowering plants suggests that the mechanisms described primarily in *A. thaliana* also control DNA methylation in other plant species.



with gene activation and restricts histone H2A.Z to the 5′ end of genes[30,34,35]. Two plant-specific DNA methyltransferases — CHROMOMETHYLASE 2 (CMT2) and CMT3 — methylate DNA in a non-CG context. CMT2 methylates both CHG and CHH contexts *de novo*[35], and CMT3 maintains CHG methylation[36]. Furthermore, a positive feedback loop enables the propagation of CHG methylation together with H3K9me2 through cell division[33] (BOX 1). The feedback loop is based on the binding of the histone H3K9 methyltransferase SU(VAR) HOMOLOGUE 4 (SUVH4; also known as KRYPTONITE) to the hemimethylated DNA in a CHG context[33], as well as on the binding of the chromodomain and the bromo adjacent homology (BAH) domain of CMT3 to H3K9me2 (REF. 37). An additional mechanism of DNA methylation is the RNA-directed DNA methylation (RdDM) pathway, which methylates DNA *de novo* in all sequence contexts, including CHH and CHG sites that are not methylated by CMT2 (REF. 29,38). RdDM involves small interfering RNAs (siRNAs), which target the *de novo* DNA methyltransferases DOMAINS REARRANGED METHYLTRANSFERASE 1 (DRM1) and DRM2 to genomic sites for DNA methylation[38]. Similar to CMT2 and CMT3, RdDM is also associated with H3K9me2 (REF. 39) (BOX 1); however, in addition to CHG and CHH methylation, DRM1 and DRM2 can also methylate DNA in CG contexts[40]. Finally, it has been shown that DNA methylation of TEs in heterochromatin depends on DECREASED DNA METHYLATION 1 (DDM1) — the SNF2 family nucleosome remodeller[41]

Endosperm
The product of the fertilized central cell. It protects the embryo, controls the transfer of nutrients from the mother and, in some species, stores seed nutrient reserves. The role of the endosperm can be compared to that of the placenta in mammals.

— which may allow DNA methyltransferases access to heterochromatin through removal of the DNA linker histone H1 (REF. 35). Hence, a range of factors act together with DNA methyltransferases to establish complex DNA methylation patterns in the plant genome.

Reprogramming through male gametogenesis

Unlike in mammals, germ lines in flowering plants do not become specified until late in their life cycle when plants produce flowers (FIG. 1). First, the male meiocyte (pollen mother cell) becomes specified from somatic cells in the male reproductive tissue of the flower[8] and initiates meiosis to produce four haploid microspores. Each microspore undergoes an asymmetrical division to produce the pollen vegetative cell (male companion cell) and the generative cell (germline cell)[12]. After the pollen has been deposited onto the stigma (female receptive organ), the vegetative cell germinates a pollen tube. The generative cell divides once more to produce two sperm cells (male gametes). Depending on the species, this last division occurs either before or after pollen tube germination. The two sperm cells and the pollen vegetative cell constitute the mature pollen grain, and immotile sperm cells in flowering plants are transferred to the female gamete via the pollen tube[42] (FIG. 1).

DNA demethylation in male meiocytes. TE expression is controlled cooperatively by H3K9me2 and non-CG methylation that depends on CMT2, CMT3 and RdDM[29]. Upregulation of TEs in *A. thaliana* male meiocytes is prominent[9,43], which suggests that DNA methylation and H3K9 methylation are reduced before meiosis. However, genome-wide profiles of epigenetic marks in meiocytes and their precursor cells are not yet available, and further studies are required to provide a mechanism for the release of TE silencing associated with meiotic precursors. There are mechanisms to prevent the retrotransposition of transcriptionally active retrotransposons[44]; thus, the release of TE silencing in meiocytes does not necessarily lead to TE transposition or genome instability. DNA methylation and H3K9me2 profiles in meiocytes are now required to further understand the epigenetic status of this important developmental phase of the plant life cycle.

DNA methylation dynamics from the microspore to the mature pollen. Genome-wide methylome profiling of sperms and pollen vegetative cells of the mature pollen indicates dynamic DNA methylation changes during male gametogenesis[45,46] (FIG. 2). In sperm cells, CG methylation remains at a comparable level to that measured in microspores, whereas CHH methylation decreases further[45]. This is consistent with the expression of MET1 and the lack of DRM2 expression (BOX 1) in sperm cells[47–49]. However, in sperm cells, the activity of other non-CG methyltransferases such as CMT2 is still unknown. In addition, levels of H3K9me2 are conserved in sperm cells[50], and this is most likely to account for the persistent silencing of TEs.

In contrast to sperm cells, CG methylation in the pollen vegetative cell decreases relative to the level observed in microspores[45]. The loss of CG methylation in the pollen vegetative cell is likely to be caused by reduced expression of MET1 (REF. 49) together with active demethylation by DME, which is not expressed in sperm cells[51]. In addition, H3K9me2 is barely detectable in the pollen vegetative cell[50]. As a likely consequence of the collective lack of CG methylation and H3K9me2, some hypomethylated TEs become mobile[52], and genes silenced by DNA methylation in somatic cells become expressed[45,46]. Unlike CG methylation, CHG methylation levels are not affected, whereas CHH methylation levels increase in the pollen vegetative cell and become comparable to or higher than those in somatic cells[45]. This results primarily from an increase of CHH methylation in the TE-rich pericentromeric regions where long terminal repeat (LTR) retrotransposons are found. Interestingly, LTR retrotransposons in the pollen vegetative cell are actively expressed, which indicates that they are not silenced by the canonical RdDM pathway and CMT2 (FIG. 2). The canonical RdDM pathway is characterized by RNA polymerase IV (Pol IV)-dependent transcripts that are converted to double-stranded RNA by RNA-DEPENDENT RNA POLYMERASE 2 (RDR2) and that are subsequently processed by DICER-LIKE 3 (DCL3) into 24-nucleotide siRNAs[27,38] (BOX 1). Indeed, low levels of 24-nucleotide siRNAs that correspond to LTR retrotransposons in the pollen vegetative cell indicate low activity of the canonical RdDM pathway. Instead, a class of 21-nucleotide siRNAs from LTR retrotransposons are likely to be enriched in the *A. thaliana* pollen vegetative cell[45,52], which suggests that a pathway distinct from the canonical RdDM pathway is involved in the gain of non-CG methylation in this cell type. DRM2 is expressed specifically in the pollen vegetative cell and not in sperm cells[45], and this supports the idea that a distinct pathway involving 21-nucleotide siRNAs guides DRM2 to methylate LTR retrotransposons[53]. The pollen vegetative cell expresses neither MET1 nor the chromatin remodeller DDM1 (FIG. 2). In the absence of DDM1 (in a *ddm1*-mutant *A. thaliana* strain), DNA methylation is lost over TEs and repeats, which are enriched in heterochromatin marked by histone H1 (REFS 35,54). In addition, LTR retrotransposons are methylated in the *ddm1* mutant by a non-canonical RdDM pathway that involves Pol II-dependent transcripts. These transcripts are converted to double-stranded RNA by RDR6 and processed into 21-nucleotide siRNAs that target DRM2 to retrotransposons[55] (BOX 1). As a result, LTR and Gypsy-type TEs become CHH hypermethylated in somatic tissues of *ddm1*- or *met1*-mutant *A. thaliana* strains[56]. Active transcription of *RDR6* and *DRM2* in the wild-type pollen vegetative cell[45,57] is likely to sustain the activity of the RDR6-dependent non-canonical RdDM pathway, which produces 21-nucleotide siRNAs and CHH hypermethylation in pericentromeric regions. The biological functions of the non-canonical RdDM pathway and CHH hypermethylation in LTR retrotransposons in the pollen vegetative cell remain unclear.

What is the developmental role of the epigenetic differences between the sperm cells and their companion

RNA-directed DNA methylation
(RdDM). A plant-specific pathway that regulates *de novo* DNA methylation in all sequence contexts (CG, CHG and CHH). Small RNAs establish DNA methylation by guiding protein components required for DNA methylation to genomic loci that are homologous to the small RNAs.

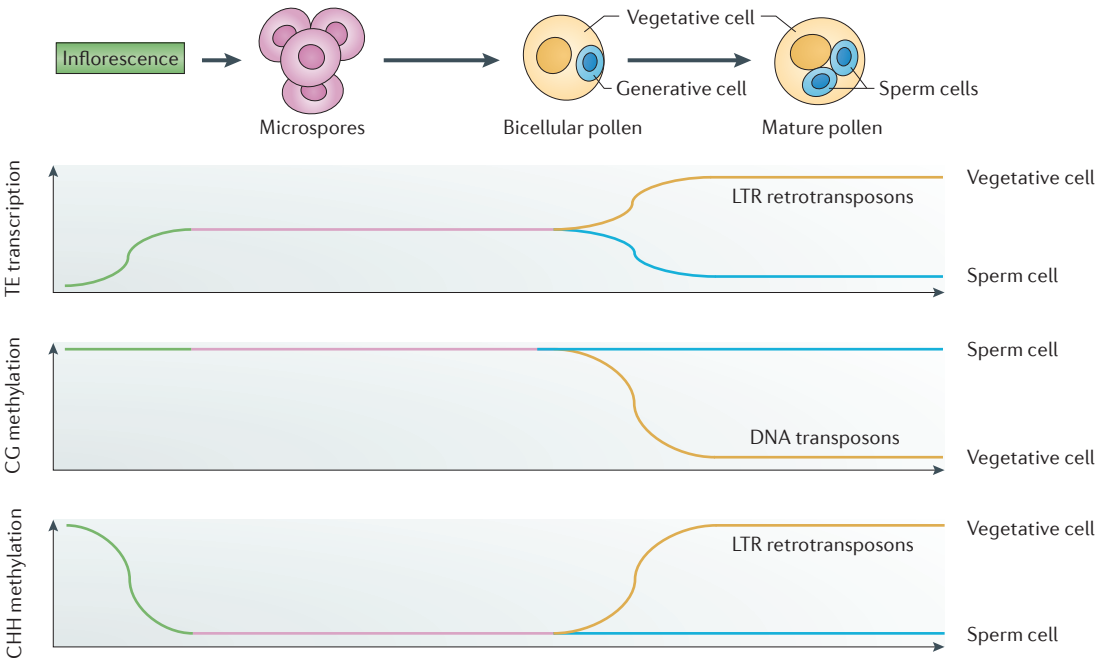
Meiocyte
The cell differentiated from the somatic cell in a position-dependent manner to undergo meiosis. Male and female meiocytes are also known as pollen mother cells and megaspore mother cells, respectively.

Asymmetrical division
Cell division that results in two cells with dissimilar morphologies and/or fates.

Pollen vegetative cell
The male companion cell generated during male gametogenesis. It germinates to give rise to the pollen tube, through which sperm cells are transferred to the female gamete.

Generative cell
The male germline cell, which undergoes one round of cell division to generate two sperm cells in the vegetative cell.

Sperm cells
Male gametes produced in the pollen.



Function	Gene	Involved in	Vegetative cell	Sperm cell
Chromatin remodelling	*DDM1*	DNA methylation in heterochromatic regions	–	+
DNA methylation	*MET1*	CG methylation	–	+
	CMT2	CHG and CHH *de novo* methylation	?	?
	CMT3	CHG methylation	–	+
	DRM2	*De novo* methylation through the RdDM pathway	+	–
DNA demethylation	DME family	DNA demethylation	+	–

Figure 2 | **Epigenetic reprogramming during male gametogenesis.** The level of CHH methylation decreases from the microspore stage until the sperm cell stage; however, the level of CG methylation remains stable, which is consistent with the expression of genes involved in DNA methylation. As it is not directly related to the level of DNA methylation, transcription of transposable elements (TEs) is prominent in the vegetative cell but not in sperm cells. In contrast to the sperm cells, the vegetative cell loses CG methylation and restores CHH methylation especially at long terminal repeat (LTR) retrotransposons. For the vegetative cell and sperm cells, the expression status of various enzymes involved in DNA methylation and demethylation is shown in the table. *CMT2*, *CHROMOMETHYLASE 2*; *DDM1*, *DECREASED DNA METHYLATION 1*; DME, DEMETER; *DRM2*, *DOMAINS REARRANGED METHYLTRANSFERASE 2*; *MET1*, *DNA METHYLTRANSFERASE 1*; RdDM, RNA-directed DNA methylation.

vegetative cell? *dme*-mutant pollen grains show abnormally high levels of CG methylation in vegetative cells and defects in viability[51], which suggests a potential advantage conferred by DME-mediated CG demethylation in the pollen vegetative cell. In addition, Slotkin *et al.*[52] showed that TEs are expressed and processed into siRNAs in the pollen vegetative cell, which coincides with silencing of TEs in the sperm cells. The authors thus proposed that siRNAs produced by active TEs in the pollen vegetative cell move to the sperm cells, where they reinforce TE silencing[52]. However, this hypothesis has been challenged by recent results. Artificial microRNAs (amiRNAs) that are expressed exclusively in the pollen vegetative cell are unable to silence a target gene expressed in sperm cells[58]. Additionally, there remains much controversy[59,60] over the presence of a direct cytoplasmic connection between sperm cells and the pollen vegetative cell provided by plasmodesmata, through which siRNAs can move between these cells[61]. Silencing of target genes by amiRNAs produced in microspores[58] provides an alternative explanation for the results obtained by Slotkin *et al.*[52]. In the wild type, it is possible that siRNAs produced by microspores are inherited upon differentiation into sperm cells, in which they target LTR retrotransposon silencing through the canonical RdDM pathway. Furthermore, after fertilization, siRNAs might be inherited from sperm cells to the zygote, in which they may have a role in reprogramming events during embryogenesis.

Reprogramming through female gametogenesis

Similarly to male germline specification, the female meiocyte (megaspore mother cell) becomes specified from a somatic precursor late in flower development[8].

After meiosis, unlike male haploid cells, only one of the four female haploid cells survives and becomes the functional megaspore (FIG. 1). In *A. thaliana,* the megaspore undergoes three rounds of nuclear division and gives rise to a syncytial female gametophyte with eight nuclei[8,15]. The eight nuclei are partitioned by cytokinesis to generate the mature embryo sac (female gametophyte) that comprises the egg cell (female gamete), the central cell (female companion cell) and accessory cells (antipodals and synergids). Thus, each female meiocyte produces a single female gametophyte in an ovule (FIG. 1). The egg cell and the central cell are each fertilized by a sperm cell to produce the zygote and the endosperm, respectively (FIG. 1). In most plant species, the central cell carries two haploid genomes, which results in a triploid endosperm after fertilization.

Female sporogenesis: the specification of the female meiocyte. In the absence of DNA methylation profiles of female meiotic precursors, meiocytes and gametes, evidence for epigenetic reprogramming originates from analyses of TE expression and from expression profiles of DNA methyltransferases and factors in associated pathways. In a striking parallel to the male meiocyte, female meiocytes in rice express TEs that are silenced in somatic tissues[62], and both transcriptional and chromatin reprogramming indeed occurs in *A. thaliana* female meiocytes[63,64]. Mutations in *A. thaliana AGO9* (which encodes an Argonaute protein) and in other genes involved in small-RNA-mediated gene silencing pathways — *SUPRESSOR OF GENE SILENCING 3* (*SGS3*), *RDR2, RDR6* and *DCL3* — cause proliferation of additional meiocytes that can initiate sporogenesis[65]. These genes are expressed in epidermal cells around the female meiocyte precursor and are expected to suppress sporogenesis in cells surrounding the female meiocyte precursor cell. In maize, mutation of an AGO homologue (*ago104*) produces functional unreduced gametes owing to aberrant meiosis[66]. In addition, the semi-dominant mutation of *A. thaliana AGO5* (*ago5-4*) causes a defect in female gametophyte development after functional megaspore generation, and this is not observed in *ago9* mutants[67]. AGO9 preferentially binds to 24-nucleotide siRNAs, whereas AGO5 has no obvious binding preferences to different sizes of siRNAs[65,68–70]; this suggests that distinct AGO5- and AGO9-dependent pathways participate in different developmental processes, such as specification of the meiocyte, meiosis and the initiation of gametogenesis. *AGO9* and *AGO5* are expressed in somatic cells that surround the female meiocyte to prevent meiotic cell fate acquisition and to support female gametogenesis, respectively, in a non-cell-autonomous manner[66,67]. On the basis of the observation of intercellular mobility of small RNAs between somatic cells[71–73], it has been proposed that mobile small RNAs are involved in the specification of female meiotic precursors and megaspores[66,67]. Plasmodesmata, which establish cytoplasmic connections, are present between the female meiocyte and the surrounding somatic cells[74], and they could facilitate the movement of small RNAs during female sporogenesis.

DNA demethylation during female gametogenesis. As a result of MET1 repression and DME activation, DNA demethylation is likely to take place in all sequence contexts in the *A. thaliana* central cell. This presumably leads to hypomethylation and transcriptional activation of any locus that is under the control of methylated *cis*-elements, including TEs and *cis*-elements that control the expression of imprinted genes. Imprinted genes are expressed at very different levels by the two parental alleles[35,75,76]; they are also found in maize and rice, and a large proportion of these genes are expressed in the endosperm[75]. Such imprinted parental expression is thought to reflect the differential levels of DNA methylation at a *cis*-element between the paternal and the maternal alleles. The archetype of imprinted expression is represented by the *A. thaliana FWA* gene[77]. CG methylation of a *cis*-element in the promoter of *FWA* silences *FWA* expression in somatic tissues. In the central cell of the female gametophyte, *FWA* methylation is removed, which leads to its expression. By contrast, in the male gamete, *FWA* methylation persists, and after fertilization the endosperm inherits a silent methylated paternal allele and an active unmethylated maternal allele. The imprinted maternal status of *FWA* is maintained in the endosperm by MET1. There are cases in which regulation of plant imprinted genes is more complex but, in all cases studied so far, DNA methylation is lost on a *cis*-element of the maternal allele, which occurs presumably in the central cell. In fact, global DNA demethylation has been measured only in the endosperm[76,78], and direct evidence of reduced DNA methylation in the central cell is still lacking. However, as DNA methylation is replicated in a semi-conservative manner and MET1 is active in the endosperm, it is possible that the loss of DNA methylation in the endosperm reflects DNA demethylation in the central cell (FIG. 3). *A. thaliana* STRUCTURE SPECIFIC RECOGNITION PROTEIN 1 (SSRP1), which is a component of the FACT (facilitates chromatin transcription/transaction) histone chaperone complex, is required for DME-dependent DNA demethylation of maternally expressed imprinted loci in the central cell[79]. Although the precise mechanism has not been uncovered, SSRP1 might enable DME to access the chromatin and to facilitate global DNA demethylation in the central cell. Nevertheless, the biological roles of DNA demethylation in the central cell remain unclear. The lack of demethylation in the central cell of *dme* mutants does not result in a loss of cell identity but impairs endosperm development as a consequence of the lack of expression of two key maternally expressed imprinted genes *FIS2* and *MEA*[47,80]. These genes encode essential subunits of Polycomb repressive complex 2 (PRC2), which regulates expression of a wide range of genes that are important for endosperm development[81,82]. The direct role of most other imprinted genes remains unknown, and there are ongoing debates about whether specific mechanisms were selected to achieve imprinting during evolution or whether imprinting in plants is a by-product of partial erasure of epigenetic marks during female gametogenesis[75,83].

Embryo sac
The female gametophyte that contains four cell types: the egg cell (female gamete), the central cell (female companion cell) and accessory cells (three antipodal cells and two synergid cells).

Egg cell
The female gamete, which produces the embryo. As the product of the fertilized egg cell reinitiates the plant life cycle, the egg cell can be considered the true female gamete.

Central cell
The female companion cell generated from female gametogenesis. It is fertilized by the sperm cell to give rise to the endosperm and can be considered the somatic part of the female gametophyte, which reinitiates its development following fertilization.

Argonaute
A family of effector proteins involved in small-RNA-directed gene silencing. Small RNAs bind to Argonaute proteins and guide the complex to their RNA targets.

Functional unreduced gametes
Gametes produced in the absence of the reduction meiotic division. They are diploid and result in triploid progeny after fertilization.

Imprinted genes
Genes in which one allele is silenced, whereas the other allele is expressed in a parent-of-origin-specific manner.

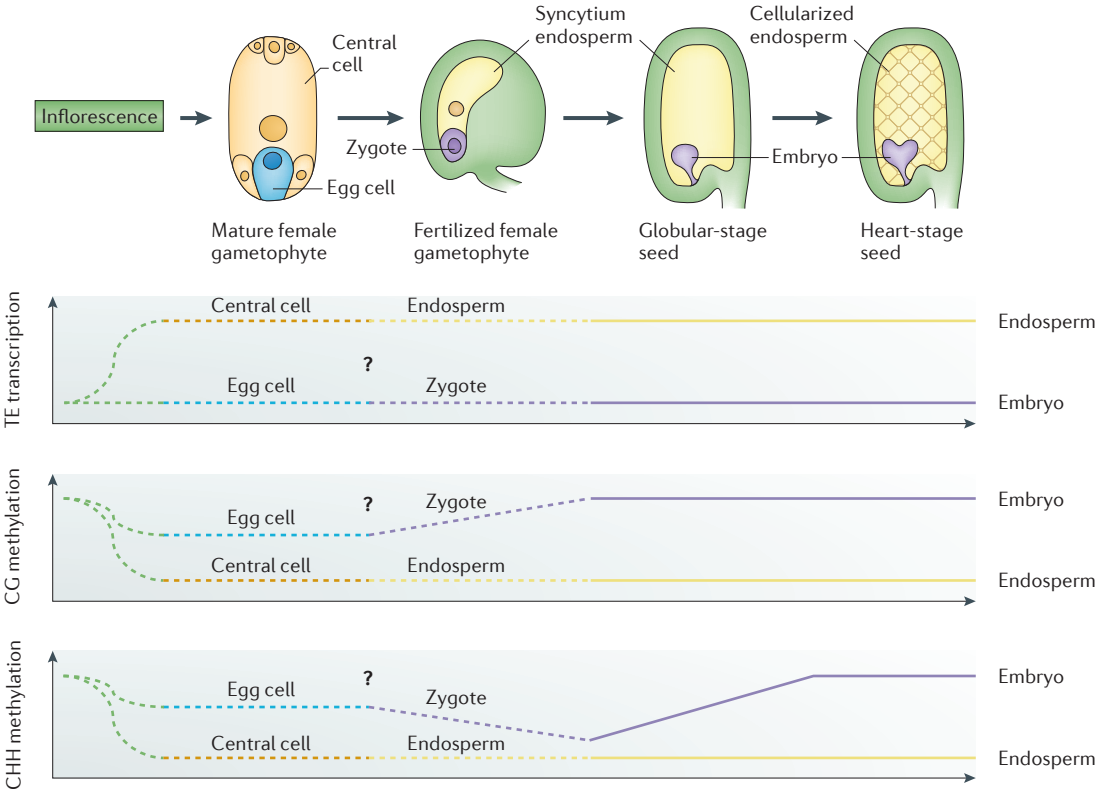


Figure 3 | **Epigenetic reprogramming during embryogenesis.** Indirect evidence (represented as dashed lines) suggests that levels of DNA methylation are very low in the central cell and reduced in the egg cell. This is reflected in the early endosperm genome, which is characterized by reduced DNA methylation levels and active transcription of transposable elements (TEs). By contrast, CG methylation in the embryo is restored to the adult level by the globular embryo stage. CHH methylation in the zygote is expected to be reduced owing to the incorporation of the CHH-demethylated paternal genome. The recovery of CHH methylation in the embryo is completed by the heart-shaped embryo stage when the endosperm becomes cellularized.

Similarly to the central cell, the egg cell expresses barely detectable amounts of the maintenance CG and CHG DNA methyltransferases, MET1 and CMT3, respectively[49] (BOX 1), which suggests that methylation levels are low in the egg cell. However, in contrast to the central cell, the egg cell does not seem to express members of the DME family[76,84] and rather expresses the *de novo* DNA methyltransferases DRM1 and DRM2 (REF. 49). DRM1 seems to be expressed only in the egg cell, which might reflect the importance of non-CG methylation in this cell type. Expression of genes related to small RNA pathways, such as *AGO* genes, is enriched in the *A. thaliana* egg cell[84], and several retrotransposons become ectopically activated in *ago9*-mutant egg cells[65]. These results suggest that RdDM pathways are still active in the egg cell and have roles in non-CG methylation for TE silencing. Additional *de novo* DNA methylation might originate from the potential activity of CMT2 (REF. 29) (BOX 1) and might balance the putative loss of CG and/or CHG methylation to maintain genome integrity in the female gamete (FIG. 3). However, direct measurement of DNA methylation in the egg cell is lacking, and this hypothesis is based on yet incomplete sets of profiles of DNA methyltransferase expression during female gametogenesis. As proposed in the case of the pollen vegetative cell, derepression of TEs in the central cell could be associated with production of siRNAs that could move to the egg cell and trigger silencing of TEs[46]. Such a mechanism to control TE expression in reproduction would be comparable to the role of PIWI-interacting RNAs (piRNAs) in animals that suppress TEs in the germ line[85]. In zebrafish and mice, piRNAs are found in both the testes and the ovaries, and a substantial proportion of these piRNAs are derived from TEs and repeats, which suggests that this pathway also silences transposons in these species[86–88]. In mice, reduced production of piRNAs compromises maintenance of the male germ line[88]. These results indicate the importance of small non-coding RNAs for reproduction in eukaryotes.

Zygotic and embryonic epigenetic reprogramming

Reprogramming of DNA methylation during early embryonic development. Fertilization of the egg cell strongly promotes the expression of genes that are involved in both CG and non-CG methylation, and these high expression levels are maintained throughout early embryogenesis[49,89]. Consequently, this leads to a

net gain in DNA methylation at specific loci and probably affects the genome-wide level of DNA methylation. Such a reprogramming event is likely to compensate for the prior loss of CG (and possibly CHG) methylation in the egg cell and the loss of CHH methylation in the sperm cell. Although genome-wide DNA methylation changes in the embryo have not been shown directly, early embryogenesis seems to be the site and time of a major reprogramming event that resets DNA methylation of the early-stage embryo to the level of somatic cells[49] (FIG. 3).

What is the functional importance of DNA methylation reprogramming after fertilization in the embryo? Putative DNA demethylation of the female gametes could trigger the expression of TEs and genes that are otherwise silenced by DNA methylation in somatic cells. Although there is no direct evidence to show DNA methylation levels and TE expression in the egg cell, experiments using a *GFP* reporter gene under the control of DNA methylation support the view that the zygote has a capacity for transcriptional repression of genes and TEs that are regulated by DNA methylation[49]. This suggests that a high level of *de novo* methylation participates in DNA methylation reprogramming in the zygote at least immediately after fertilization and contributes to the maintenance of genome integrity in the embryo.

Beyond the upregulation of methyltransferases, the mechanisms and impact associated with reprogramming of DNA methylation are an area of interest and debate. As the *A. thaliana* egg cell does not express DME and markers of DNA methylation such as imprinted genes, it is possible that the egg cell undergoes only a limited degree of loss of DNA methylation compared to somatic levels, which has a minor impact on TE expression and genome integrity. Alternatively, other mechanisms might silence TEs and imprinted genes in spite of demethylation of DNA in the egg cell.

During embryonic development, it has been proposed that siRNAs may be produced in the endosperm and transferred to the embryo[46]. Indeed, there is emerging evidence for communication between the embryo and the endosperm that involves kinase signalling pathways[90,91]. However, another recent study showed that the early (syncytial) endosperm accumulates only low levels of mRNAs that encode RdDM pathway components, and that their expression levels increase and peak during the cellularization phase of the endosperm[89]. Endosperm cellularization occurs after the heart-shaped embryo stage in *A. thaliana* (FIG. 3), and non-coding RNAs have indeed been shown to be also expressed in this late-stage endosperm in both *A. thaliana* and rice[78,92]. If transferred to the embryo, siRNAs from the endosperm would then be expected to cause a further increase in DNA methylation in the embryo following endosperm cellularization. However, this is not the case, and DNA methylation levels in the embryo are stable after endosperm cellularization[49]. Moreover, the zygote and the young embryo already express high levels of several RdDM genes[89]. The RdDM genes that are active in the early-stage embryo are responsible for the increase of CHH methylation at least at a few loci[49] and, in theory, RdDM activity might be sufficient to enable the silencing of TEs in a cell-autonomous manner. Nevertheless, further investigations are still needed to discriminate between cell-autonomous and non-cell-autonomous silencing of TEs in the embryo.

Reconciling embryonic reprogramming of DNA methylation with inheritance of epialleles. Although the degree of reprogramming of DNA methylation levels in plants is unlikely to be as high as that reported in mammals, the observed net gain in DNA methylation and epigenetic reprogramming in the plant embryo was unexpected[49] because silencing of some loci through DNA methylation was known to be maintained through generations, and this had led to the assumption that there is a lack of resetting of DNA methylation in plants[23]. Hence, although DNA demethylation in the germ line (that is, the gametes in plants) is followed by active methylation in the plant embryo after fertilization, some sequences retain a sufficient level of DNA methylation to be transmitted to the next generation.

Indeed, a potential explanation for the occurrence of both epigenetic reprogramming and the transmission of epialleles is that the epigenome of an individual plant is determined by an extensive, albeit incomplete, reprogramming during embryonic development in addition to a partial contribution from the residual parental epigenetic states. A possible underlying mechanism of the transmission of parental epigenetic states involves the siRNAs produced by feedback loops (BOX 1). siRNAs can initiate *de novo* DNA methylation in the resultant embryo and might contribute to transgenerational inheritance of DNA methylation patterns, as shown for silenced transgenes or newly inserted TEs[93]. In support of this hypothesis, *A. thaliana met1* or *ddm1* mutants, which are largely deprived of CG methylation, show a gradual remethylation of the genome when outcrossed with wild-type plants[94,95]. Such remethylation depends on the canonical RdDM pathway and takes place over several generations until a wild-type somatic level of DNA methylation is reached[95]. This occurs in a step-wise manner from one generation to the next, which suggests that each sexual reproductive cycle causes an increase in DNA methylation[95]. This finding provides evidence that epigenetic states are determined through a combination of embryonic reprogramming and transgenerational inheritance.

Histone replacement in zygotic resetting and activation. Histone modifications such as H3K27me2 and H3K27me3 may be partially inherited through cell divisions[96]. When H3K27 methylation is perturbed, the sporophyte acquires gametophytic traits in the moss *Physcomitrella patens*[97] and to some extent in *A. thaliana*[98]. Hence, reprogramming of histone marks may underlie the transition between the gametophytic life and the sporophytic life, and is thus expected to take place at fertilization. This reprogramming mechanism in the zygote may involve specific enzymes that add or remove histone marks. However, a more global mechanism

Epialleles
Alleles that cause changes in gene expression and that are produced by epigenetic marks (generally DNA methylation in a CG context) but not by mutations in the DNA sequence.

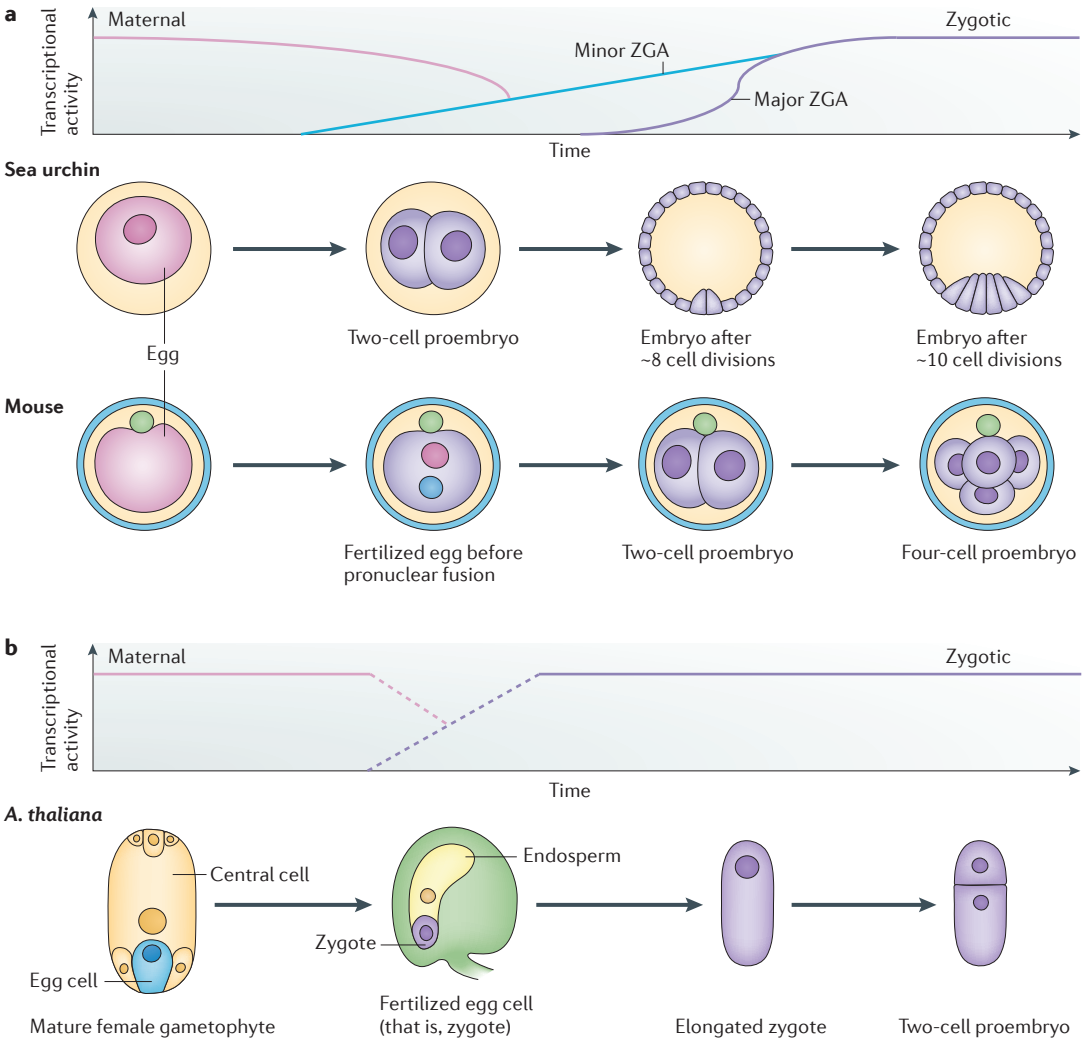
Sporophyte
The diploid life form in which meiosis takes place to produce the haploid spores.

Box 2 | **Maternal-to-zygotic transition in flowering plants**

Animal eggs become activated by the entry of sperms to complete the suspended meiosis[121]. Zygotes then undergo rapid divisions without changing the overall size, which are regulated by the stored maternal factors (see the figure, part **a**). Both minor zygotic genome activation (ZGA) and the degradation of maternal factors take place, followed by the major ZGA to switch the developmental control from maternal factors to *de novo* transcripts that are expressed from the zygotic genome.

Unlike in animals, fertilization in plants immediately triggers elongation of the zygote followed by asymmetrical cell division, which gives rise to two daughter cells with distinct cell fates[122,123]. Moreover, flowering plants undergo the sporophytic-to-multicellular-gametophytic transition for sexual reproduction (FIG. 1); these structural and developmental differences suggest that the mechanism involved in maternal-to-zygotic transition in plants might differ from that in animals. A genomic overview of *Arabidopsis thaliana* mutations shows that many mutations that result in defects early in seed development are recessive, which implies that the expression of either maternal- or paternal-derived zygotic alleles is sufficient for early embryonic development[124]. Furthermore, direct measurements of transcription in zygotes show that parental alleles are transcribed *de novo* from several loci[107,125–128], which shows that flowering plants initiate gene activation in the zygote immediately after fertilization (see the figure, part **b**).

Single-nucleotide polymorphisms between different ecotypes of a plant species can be used to distinguish which of the zygotic transcripts of an intercross are from the paternally versus maternally inherited chromosomes. Transcripts profiling on early-stage embryos from such a cross verified the presence of paternal transcripts at a genome-wide level; however, the ratios of maternal transcripts to paternal transcripts vary in different experiments[129,130]. The nature of these experiments must be carefully considered when drawing general conclusions about the relative activity of transcription from the maternal and paternal chromosomes. Although the mechanism of hybrid vigour (also known as heterosis)[131] is not known, it affects transcription and might also introduce biases in transcriptional profiles from hybrid embryos. In addition, the maternal mRNAs inherited by the zygote from the egg should not be ignored owing to difficulty in distinguishing them from the *de novo* transcripts from the maternal chromosome of the zygotic genome. Overall, although the degree of plant ZGA is unclear, flowering plants activate both maternal and paternal genomes in the zygote immediately after fertilization (see the figure, part **b**). This ZGA represents the gametophytic-to-sporophytic transition in plants[123] and might also be linked to histone variant replacement, which simultaneously takes place immediately after fertilization for reprogramming.



Hybrid vigour
A phenomenon that causes the hybrid progeny to differ from the predicted average of the parental traits.

that can simultaneously reset multiple histone marks in a single step is nucleosome replacement by newly deposited histones, which might be efficient for reprogramming in the zygote in the absence of DNA replication. Such a mechanism relies on histone variants that are distinguished from their regular histone counterparts by a few amino acid differences[99,100]. In animals, spermatozoa nuclei are devoid of histones, which are replaced by protamines. At fertilization, copies of the H3 variant H3.3 are extensively incorporated into the paternal genome, and this is essential for the onset of embryonic life[101–104]. In plants, sperm cells do not replace histones with protamines but with sperm-specific histones[12]. At fertilization, the sperm-specific histones are removed and replaced with the histone variant H3.3, which leads to reprogramming of the composition of the zygotic chromatin[105] (BOX 2).

In addition to reprogramming histone marks, another likely role for histone replacement is the maintenance of specialized centromeric heterochromatin, which is required for kinetochore formation and cell division in the zygote immediately after fertilization. Centromeres are occupied by nucleosomes that contain the centromeric variant of histone H3 (CENH3 in *A. thaliana* and centromere protein A (CENPA) in mammals)[106]. In *A. thaliana*, CENH3 is not expressed in the female gamete, and CENH3 inherited from the male gamete is lost after the fusion of the two gamete nuclei at the same time as the removal of other H3 variants that are inherited from the parents[100,107]. Hence, *de novo* CENH3 deposition is required in the zygote. Mechanisms that lead to CENH3 deposition are not known in *A. thaliana*, but CENH3 deposition generally results from the function of specific chaperones[108]. In *Drosophila melanogaster*, deposition of CenH3 (also known as Cid) is not defined by the actual DNA sequence at the centromere but by the presence of pericentromeric heterochromatin marked by H3K9me2 (REF. 109). It is likely that in *A. thaliana,* re-establishment of pericentromeric heterochromatin is crucial for the recruitment of CENH3 and the establishment of kinetochore before the first zygotic mitosis.

Conclusions and perspectives

The evolution of the plant life cycle with its unique sporophytic-to-gametophytic transition indicates that caution should be taken when comparing epigenetic and transcriptional dynamics between plants and animals. Nonetheless, in all eukaryotes, fertilization fuses two highly differentiated gametes to give rise to a totipotent zygote and involves comparable epigenetic reprogramming events. Unresolved issues related to reprogramming during gametogenesis include the origin of siRNAs that are involved in reprogramming and whether they act in a non-cell-autonomous manner. To answer these questions, a major limiting factor is currently the difficulty in isolating sufficient amounts of pure plant gametes, zygotes and early endosperm to directly identify the siRNAs and to measure the activity of the DNA methylation pathways. An alternative strategy may come from complex genetic manipulations that will enable the alteration of DNA methylation and siRNA biogenesis pathways independently in gametes, the zygote and the endosperm to address the issues of cell autonomy. Additionally, several points need to be investigated: the link between the cell cycle and heterochromatin formation at fertilization, the impact of epigenetic mechanisms on centromere function and the general implications of reprogramming in evolutionary terms.

The maternal-to-zygotic transition in plants (BOX 2) is accompanied by the reactivation of the cell cycle, which leads to the first division of the zygote. In yeast, the link between the cell cycle and heterochromatin formation is mainly achieved by cell-cycle-dependent transcriptional control of genes that modify chromatin states. In addition, the activation of positive feedback loops between the production of siRNAs and the deposition of H3K9me2 (REFS 110,111) ensures that the chromatin state is maintained after deposition of the new nucleosome following DNA replication. In plants, it remains largely unclear whether the cell cycle machinery is also linked to H3K9me2 and DNA methylation. *MET1* expression is under the control of the retinoblastoma protein[48] (the activity of which is regulated by the cell cycle), but it is not known whether such a control extends to genes encoding other DNA methyltransferases and H3K9 methyltransferase.

What are the consequences of DNA methylation reprogramming in the plant embryo? The cycle of fluctuation of DNA methylation levels between somatic cells and gametes involves loss of CG methylation and gain of CHH methylation through *de novo* DNA methylation. This alternation is predicted to cause slight changes in DNA methylation pattern, as *de novo* methylation has the potential to create new sites of methylation that did not exist in the parents. Such fluctuations of genome-wide DNA methylation patterns between generations have been observed through a genomic survey of DNA methylation profiles across individual plants that represent a lineage of up to 30 generations[112,113]. Additional epigenetic changes also occur in response to environmental changes such as temperature, dietary components and chemical pollutants[114]. Occasionally, new epigenetic marks affect a site that influences gene expression either directly by regulating the activity of nearby genes or indirectly by mobilizing TEs that can alter gene expression at their new integration sites[93]. Such a mechanism can potentially generate new DNA methylation sites in a particular context, which subsequently becomes under the control of DNA methylation maintenance. These alterations that affect gene expression may result in heritable phenotypic changes that contribute to adaptation to environmental conditions[115]. This plant-specific but relaxed mode of epigenetic reprogramming in the embryo might be rather advantageous for plants, which cannot move but need to adapt to changing environments. In the current context of increased fluctuation in weather conditions and their detrimental impact on agriculture, further research in plant epigenetics is highly important to enable selection of improved crops.

1. Hathaway, N. A. *et al.* Dynamics and memory of heterochromatin in living cells. *Cell* **149**, 1447–1460 (2012).
2. Law, J. A. & Jacobsen, S. E. Dynamic DNA methylation. *Science* **323**, 1568–1569 (2009).
3. Sharif, J. & Koseki, H. Recruitment of Dnmt1 roles of the SRA protein Np95 (Uhrf1) and other factors. *Prog. Mol. Biol. Transl. Sci.* **101**, 289–310 (2011).
4. Greer, E. L. & Shi, Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nature Rev. Genet.* **13**, 343–357 (2012).
5. Martin, C. & Zhang, Y. Mechanisms of epigenetic inheritance. *Curr. Opin. Cell Biol.* **19**, 266–272 (2007).
6. Hajkova, P. Epigenetic reprogramming in the germline: towards the ground state of the epigenome. *Phil. Trans. R. Soc. B.* **366**, 2266–2273 (2011).
7. Kota, S. K. & Feil, R. Epigenetic transitions in germ cell development and meiosis. *Dev. Cell* **19**, 675–686 (2010).
8. Berger, F. & Twell, D. Germline specification and function in plants. *Annu. Rev. Plant Biol.* **62**, 461–484 (2011).
9. Chen, C. *et al.* Meiosis-specific gene discovery in plants: RNA-seq applied to isolated *Arabidopsis* male meiocytes. *BMC Plant Biol.* **10**, 280 (2010).
10. Crismani, W., Girard, C. & Mercier, R. Tinkering with meiosis. *J. Exp. Bot.* **64**, 55–65 (2013).
11. Brownfield, L. & Kohler, C. Unreduced gamete formation in plants: mechanisms and prospects. *J. Exp. Bot.* **62**, 1659–1668 (2011).
12. Twell, D. Male gametogenesis and germline specification in flowering plants. *Sex. Plant Reprod.* **24**, 149–160 (2011).
13. Palovaara, J., Saiga, S. & Weijers, D. Transcriptomics approaches in the early *Arabidopsis* embryo. *Trends Plant Sci.* **18**, 514–521 (2013).
14. Li, J. & Berger, F. Endosperm: food for humankind and fodder for scientific discoveries. *New Phytol.* **195**, 290–305 (2012).
15. Drews, G. N. & Koltunow, A. M. The female gametophyte. *Arabidopsis Book* **9**, e0155 (2011).
16. Leitch, H. G., Tang, W. W. & Surani, M. A. Primordial germ-cell development and epigenetic reprogramming in mammals. *Curr. Top. Dev. Biol.* **104**, 149–187 (2013).
17. Seisenberger, S. *et al.* Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. *Phil. Trans. R. Soc. B* **368**, 20110330 (2013).
18. Cantone, I. & Fisher, A. G. Epigenetic programming and reprogramming during development. *Nature Struct. Mol. Biol.* **20**, 282–289 (2013).
19. Chedin, F. The DNMT3 family of mammalian *de novo* DNA methyltransferases. *Prog. Mol. Biol. Transl. Sci.* **101**, 255–285 (2011).
20. Smallwood, S. A. & Kelsey, G. *De novo* DNA methylation: a germ cell perspective. *Trends Genet.* **28**, 33–42 (2012).
21. Cubas, P., Vincent, C. & Coen, E. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* **401**, 157–161 (1999).
22. Hauser, M. T., Aufsatz, W., Jonak, C. & Luschnig, C. Transgenerational epigenetic inheritance in plants. *Biochim. Biophys.* **1809**, 459–468 (2011).
23. Saze, H., Mittelsten Scheid, O. & Paszkowski, J. Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. *Nature Genet.* **34**, 65–69 (2003).
24. Gehring, M. *et al.* DEMETER DNA glycosylase establishes *MEDEA* Polycomb gene self-imprinting by allele-specific demethylation. *Cell* **124**, 495–506 (2006).
25. Gong, Z. *et al. ROS1*, a repressor of transcriptional gene silencing in *Arabidopsis*, encodes a DNA glycosylase/lyase. *Cell* **111**, 803–814 (2002).
26. Castel, S. E. & Martienssen, R. A. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nature Rev. Genet.* **14**, 100–112 (2013).
27. Haag, J. R. & Pikaard, C. S. Multisubunit RNA polymerases IV and V: purveyors of non-coding RNA for plant gene silencing. *Nature Rev. Mol. Cell. Biol.* **12**, 483–492 (2011).
28. Law, J. A. & Jacobsen, S. E. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nature Rev. Genet.* **11**, 204–220 (2010).
29. Stroud, H. *et al.* Non-CG methylation patterns shape the epigenetic landscape in *Arabidopsis*. *Nature Struct. Mol. Biol.* **21**, 64–72 (2014).
This paper provides a comprehensive account of all DNA methyltransferase activities that are responsible for non-CG methylation in *A. thaliana*.
30. Cokus, S. J. *et al.* Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* **452**, 215–219 (2008).
31. Zemach, A., McDaniel, I. E., Silva, P. & Zilberman, D. Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* **328**, 916–919 (2010).
32. Finnegan, E. J., Peacock, W. J. & Dennis, E. S. Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proc. Natl Acad. Sci. USA* **93**, 8449–8454 (1996).
33. Johnson, L. M. *et al.* The SRA methyl-cytosine-binding domain links DNA and histone methylation. *Curr. Biol.* **17**, 379–384 (2007).
34. Coleman-Derr, D. & Zilberman, D. DNA methylation, H2A.Z, and the regulation of constitutive expression. *Cold Spring Harb. Symp. Quant. Biol.* **77**, 147–154 (2012).
35. Zemach, A. *et al.* The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* **153**, 193–205 (2013).
36. Lindroth, A. M. *et al.* Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. *Science* **292**, 2077–2080 (2001).
37. Du, J. *et al.* Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants. *Cell* **151**, 167–180 (2012).
38. Matzke, M. A. & Mosher, R. A. RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. *Nature Rev. Genet.* **15**, 394–408 (2014).
39. Law, J. A., Vashisht, A. A., Wohlschlegel, J. A. & Jacobsen, S. E. SHH1, a homeodomain protein required for DNA methylation, as well as RDR2, RDM4, and chromatin remodeling factors, associate with RNA polymerase IV. *PLoS Genet.* **7**, e1002195 (2011).
40. Zhong, X. *et al.* Molecular mechanism of action of plant DRM *de novo* DNA methyltransferases. *Cell* **157**, 1050–1060 (2014).
41. Brzeski, J. & Jerzmanowski, A. Deficient in DNA methylation 1 (DDM1) defines a novel family of chromatin-remodeling factors. *J. Biol. Chem.* **278**, 823–828 (2003).
42. Kawashima, T. & Berger, F. Green love talks; cell–cell communication during double fertilization in flowering plants. *AoB Plants* **2011**, plr015 (2011).
43. Yang, H., Lu, P., Wang, Y. & Ma, H. The transcriptome landscape of *Arabidopsis* male meiocytes from high-throughput sequencing: the complexity and evolution of the meiotic process. *Plant J.* **65**, 503–516 (2011).
44. Ito, H. *et al.* An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature* **472**, 115–119 (2011).
45. Calarco, J. P. *et al.* Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell* **151**, 194–205 (2012).
This study provides evidence for dynamic changes of DNA methylation during male gametogenesis in *A. thaliana*.
46. Ibarra, C. A. *et al.* Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science* **337**, 1360–1364 (2012).
This paper shows demethylation in plant gamete companion cells.
47. Jullien, P. E., Kinoshita, T., Ohad, N. & Berger, F. Maintenance of DNA methylation during the *Arabidopsis* life cycle is essential for parental imprinting. *Plant Cell* **18**, 1360–1372 (2006).
48. Jullien, P. E. *et al.* Retinoblastoma and its binding partner MSI1 control imprinting in *Arabidopsis*. *PLoS Biol.* **6**, e194 (2008).
49. Jullien, P. E., Susaki, D., Yelagandula, R., Higashiyama, T. & Berger, F. DNA methylation dynamics during sexual reproduction in *Arabidopsis thaliana*. *Curr. Biol.* **22**, 1825–1830 (2012).
This study reports the first evidence for a cycle of DNA methylation reprogramming at specific loci in *A. thaliana*.
50. Schoft, V. K. *et al.* Induction of RNA-directed DNA methylation upon decondensation of constitutive heterochromatin. *EMBO Rep.* **10**, 1015–1021 (2009).
51. Schoft, V. K. *et al.* Function of the DEMETER DNA glycosylase in the *Arabidopsis thaliana* male gametophyte. *Proc. Natl Acad. Sci. USA* **108**, 8042–8047 (2011).
52. Slotkin, R. K. *et al.* Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* **136**, 461–472 (2009).
53. Martinez, G. & Slotkin, R. K. Developmental relaxation of transposable element silencing in plants: functional or byproduct? *Curr. Opin. Plant Biol.* **15**, 496–502 (2012).
54. Lippman, Z. *et al.* Role of transposable elements in heterochromatin and epigenetic control. *Nature* **430**, 471–476 (2004).
55. Nuthikattu, S. *et al.* The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21–22 nucleotide small interfering RNAs. *Plant Physiol.* **162**, 116–131 (2013).
56. Stroud, H., Greenberg, M. V., Feng, S., Bernatavichute, Y. V. & Jacobsen, S. E. Comprehensive analysis of silencing mutants reveals complex regulation of the *Arabidopsis* methylome. *Cell* **152**, 352–364 (2013).
This is a global analysis of all pathways that control DNA methylation and their interaction with other silencing mechanisms.
57. Grant-Downton, R., Hafidh, S., Twell, D. & Dickinson, H. G. Small RNA pathways are present and functional in the angiosperm male gametophyte. *Mol. Plant* **2**, 500–512 (2009).
58. Grant-Downton, R. *et al.* Artificial microRNAs reveal cell-specific differences in small RNA activity in pollen. *Curr. Biol.* **23**, R599–R601 (2013).
This report provides a new insight into the controversy related to the movement of non-coding RNAs in pollen.
59. Russell, S. D. Ultrastructure of the sperm of plumbago-zeylanica: II. Quantitative cytology and 3-dimensional organization. *Planta* **162**, 385–391 (1984).
60. McCue, A. D., Cresti, M., Feijo, J. A. & Slotkin, R. K. Cytoplasmic connection of sperm cells to the pollen vegetative cell nucleus: potential roles of the male germ unit revisited. *J. Exp. Bot.* **62**, 1621–1631 (2011).
61. Kragler, F. Plasmodesmata: intercellular tunnels facilitating transport of macromolecules in plants. *Cell Tissue Res.* **352**, 49–58 (2013).
62. Kubo, T. *et al.* Transcriptome analysis of developing ovules in rice isolated by laser microdissection. *Plant Cell Physiol.* **54**, 750–765 (2013).
63. Schmidt, A. *et al.* Transcriptome analysis of the *Arabidopsis* megaspore mother cell uncovers the importance of RNA helicases for plant germline development. *PLoS Biol.* **9**, e1001155 (2011).
64. She, W. *et al.* Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. *Development* **140**, 4008–4019 (2013).
65. Olmedo-Monfil, V. *et al.* Control of female gamete formation by a small RNA pathway in *Arabidopsis*. *Nature* **464**, 628–632 (2010).
66. Singh, M. *et al.* Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein. *Plant Cell* **23**, 443–458 (2011).
67. Tucker, M. R. *et al.* Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in *Arabidopsis*. *Development* **139**, 1399–1404 (2012).
68. Borges, F., Pereira, P. A., Slotkin, R. K., Martienssen, R. A. & Becker, J. D. MicroRNA activity in the *Arabidopsis* male germline. *J. Exp. Bot.* **62**, 1611–1620 (2011).
69. Mi, S. *et al.* Sorting of small RNAs into *Arabidopsis* argonaute complexes is directed by the 5′ terminal nucleotide. *Cell* **133**, 116–127 (2008).
70. Takeda, A., Iwasaki, S., Watanabe, T., Utsumi, M. & Watanabe, Y. The mechanism selecting the guide strand from small RNA duplexes is different among argonaute proteins. *Plant Cell Physiol.* **49**, 493–500 (2008).
71. Dunoyer, P. *et al.* Small RNA duplexes function as mobile silencing signals between plant cells. *Science* **328**, 912–916 (2010).
72. Melnyk, C. W., Molnar, A., Bassett, A. & Baulcombe, D. C. Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. *Curr. Biol.* **21**, 1678–1683 (2011).
73. Molnar, A. *et al.* Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* **328**, 872–875 (2010).
74. Bajon, C., Horlow, C., Motamayor, J. C., Sauvanet, A. & Robert, D. Megasporogenesis in *Arabidopsis thaliana* L.: an ultrastructural study. *Sex. Plant Reprod.* **12**, 99–109 (1999).
75. Gehring, M. Genomic imprinting: insights from plants. *Annu. Rev. Genet.* **47**, 187–208 (2013).
76. Gehring, M., Bubb, K. L. & Henikoff, S. Extensive demethylation of repetitive elements during seed development underlies gene imprinting. *Science* **324**, 1447–1451 (2009).

77. Kinoshita, T. *et al.* One-way control of *FWA* imprinting in *Arabidopsis* endosperm by DNA methylation. *Science* **303**, 521–523 (2004).
78. Hsieh, T. F. *et al.* Genome-wide demethylation of *Arabidopsis* endosperm. *Science* **324**, 1451–1454 (2009).
79. Ikeda, Y. *et al.* HMG domain containing SSRP1 is required for DNA demethylation and genomic imprinting in *Arabidopsis*. *Dev. Cell* **21**, 589–596 (2011).
80. Choi, Y. *et al.* DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in *Arabidopsis*. *Cell* **110**, 33–42 (2002).
81. Ingouff, M., Haseloff, J. & Berger, F. Polycomb group genes control developmental timing of endosperm. *Plant J.* **42**, 663–674 (2005).
82. Wolff, P. *et al.* High-resolution analysis of parent-of-origin allelic expression in the *Arabidopsis* endosperm. *PLoS Genet.* **7**, e1002126 (2011).
83. Berger, F., Vu, T. M., Li, J. & Chen, B. Hypothesis: selection of imprinted genes is driven by silencing deleterious gene activity in somatic tissues. *Cold Spring Harb. Symp. Quant. Biol.* **77**, 23–29 (2012).
84. Wuest, S. E. *et al. Arabidopsis* female gametophyte gene expression map reveals similarities between plant and animal gametes. *Curr. Biol.* **20**, 506–512 (2010).
85. Ishizu, H., Siomi, H. & Siomi, M. C. Biology of PIWI-interacting RNAs: new insights into biogenesis and function inside and outside of germlines. *Genes Dev.* **26**, 2361–2373 (2012).
86. Aravin, A. *et al.* A novel class of small RNAs bind to MILI protein in mouse testes. *Nature* **442**, 203–207 (2006).
87. Houwing, S. *et al.* A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in zebrafish. *Cell* **129**, 69–82 (2007).
88. Watanabe, T. *et al.* Identification and characterization of two novel classes of small RNAs in the mouse germline: retrotransposon-derived siRNAs in oocytes and germline small RNAs in testes. *Genes Dev.* **20**, 1732–1743 (2006).
89. Belmonte, M. F. *et al.* Comprehensive developmental profiles of gene activity in regions and subregions of the *Arabidopsis* seed. *Proc. Natl Acad. Sci. USA* **110**, E435–E444 (2013).
This paper presents an 'atlas' of gene expression profiles in the major components of *A. thaliana* developing seeds, which provides new insights into the gene activity that regulates DNA methylation.
90. Costa, L. M. *et al.* Central cell-derived peptides regulate early embryo patterning in flowering plants. *Science* **344**, 168–172 (2014).
91. Xing, Q. *et al.* ZHOUPI controls embryonic cuticle formation via a signalling pathway involving the subtilisin protease ABNORMAL LEAF-SHAPE1 and the receptor kinases GASSHO1 and GASSHO2. *Development* **140**, 770–779 (2013).
92. Rodrigues, J. A. *et al.* Imprinted expression of genes and small RNA is associated with localized hypomethylation of the maternal genome in rice endosperm. *Proc. Natl Acad. Sci. USA* **110**, 7934–7939 (2013).
93. Mari-Ordonez, A. *et al.* Reconstructing *de novo* silencing of an active plant retrotransposon. *Nature Genet.* **45**, 1029–1039 (2013).
This elegant work shows that TEs can mobilize to new loci and create *de novo* epialleles in *A. thaliana*, which causes genome diversification and provides a potential source of adaptive traits.
94. Reinders, J. *et al.* Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev.* **23**, 939–950 (2009).
95. Teixeira, F. K. *et al.* A role for RNAi in the selective correction of DNA methylation defects. *Science* **323**, 1600–1604 (2009).
96. Probst, A. V., Dunleavy, E. & Almouzni, G. Epigenetic inheritance during the cell cycle. *Nature Rev. Mol. Cell. Biol.* **10**, 192–206 (2009).
97. Okano, Y. *et al.* A Polycomb repressive complex 2 gene regulates apogamy and gives evolutionary insights into early land plant evolution. *Proc. Natl Acad. Sci. USA* **106**, 16321–16326 (2009).
98. Mosquna, A. *et al.* Regulation of stem cell maintenance by the Polycomb protein FIE has been conserved during land plant evolution. *Development* **136**, 2433–2444 (2009).
99. Filipescu, D., Szenker, E. & Almouzni, G. Developmental roles of histone H3 variants and their chaperones. *Trends Genet.* **29**, 630–640 (2013).
100. Ingouff, M. & Berger, F. Histone3 variants in plants. *Chromosoma* **119**, 27–33 (2010).
101. Akiyama, T., Suzuki, O., Matsuda, J. & Aoki, F. Dynamic replacement of histone H3 variants reprograms epigenetic marks in early mouse embryos. *PLoS Genet.* **7**, e1002279 (2011).
102. Banaszynski, L. A., Allis, C. D. & Lewis, P. W. Histone variants in metazoan development. *Dev. Cell* **19**, 662–674 (2010).
103. Orsi, G. A. *et al. Drosophila* Yemanuclein and HIRA cooperate for *de novo* assembly of H3.3-containing nucleosomes in the male pronucleus. *PLoS Genet.* **9**, e1003285 (2013).
104. Santenard, A. *et al.* Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. *Nature Cell Biol.* **12**, 853–862 (2010).
105. Ingouff, M. *et al.* Zygotic resetting of the HISTONE 3 variant repertoire participates in epigenetic reprogramming in *Arabidopsis*. *Curr. Biol.* **20**, 2137–2143 (2010).
This paper provides evidence that H3 inherited from chromatin in gametes are removed from the zygotic chromatin by *de novo* synthesized H3, which suggests reprogramming of chromatin marks after fertilization.
106. Dalal, Y., Furuyama, T., Vermaak, D. & Henikoff, S. Structure, dynamics, and evolution of centromeric nucleosomes. *Proc. Natl Acad. Sci. USA* **104**, 15974–15981 (2007).
107. Aw, S. J., Hamamura, Y., Chen, Z., Schnittger, A. & Berger, F. Sperm entry is sufficient to trigger division of the central cell but the paternal genome is required for endosperm development in *Arabidopsis*. *Development* **137**, 2683–2690 (2010).
108. Stellfox, M. E., Bailey, A. O. & Foltz, D. R. Putting CENP-A in its place. *Cell. Mol. Life Sci.* **70**, 387–406 (2013).
109. Olszak, A. M. *et al.* Heterochromatin boundaries are hotspots for *de novo* kinetochore formation. *Nature Cell Biol.* **13**, 799–808 (2011).
110. Zaratiegui, M. *et al.* RNAi promotes heterochromatic silencing through replication-coupled release of RNA Pol II. *Nature* **479**, 135–138 (2011).
111. Zaratiegui, M. *et al.* CENP-B preserves genome integrity at replication forks paused by retrotransposon LTR. *Nature* **469**, 112–115 (2011).
112. Becker, C. *et al.* Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature* **480**, 245–249 (2011).
113. Schmitz, R. J. *et al.* Transgenerational epigenetic instability is a source of novel methylation variants. *Science* **334**, 369–373 (2011).
114. Feil, R. & Fraga, M. F. Epigenetics and the environment: emerging patterns and implications. *Nature Rev. Genet.* **13**, 97–109 (2011).
115. Becker, C. & Weigel, D. Epigenetic variation: origin and transgenerational inheritance. *Curr. Opin. Plant Biol.* **15**, 562–567 (2012).
116. Woo, H. R., Dittmer, T. A. & Richards, E. J. Three SRA-domain methylcytosine-binding proteins cooperate to maintain global CpG methylation and epigenetic silencing in *Arabidopsis*. *PLoS Genet.* **4**, e1000156 (2008).
117. Woo, H. R., Pontes, O., Pikaard, C. S. & Richards, E. J. VIM1, a methylcytosine-binding protein required for centromeric heterochromatinization. *Genes Dev.* **21**, 267–277 (2007).
118. Jackson, J. P., Lindroth, A. M., Cao, X. & Jacobsen, S. E. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560 (2002).
119. Malagnac, F., Bartee, L. & Bender, J. An *Arabidopsis* SET domain protein required for maintenance but not establishment of DNA methylation. *EMBO J.* **21**, 6842–6852 (2002).
120. Law, J. A. *et al.* Polymerase IV occupancy at RNA-directed DNA methylation sites requires SHH1. *Nature* **498**, 385–389 (2013).
121. Tadros, W. & Lipshitz, H. D. The maternal-to-zygotic transition: a play in two acts. *Development* **136**, 3033–3042 (2009).
122. Kawashima, T. & Goldberg, R. B. The suspensor: not just suspending the embryo. *Trends Plant Sci.* **15**, 23–30 (2010).
123. Lau, S., Slane, D., Herud, O., Kong, J. & Jurgens, G. Early embryogenesis in flowering plants: setting up the basic body pattern. *Annu. Rev. Plant Biol.* **63**, 483–506 (2012).
124. Muralla, R., Lloyd, J. & Meinke, D. Molecular foundations of reproductive lethality in *Arabidopsis thaliana*. *PLoS ONE* **6**, e28398 (2011).
125. Ingouff, M., Hamamura, Y., Gourgues, M., Higashiyama, T. & Berger, F. Distinct dynamics of HISTONE3 variants between the two fertilization products in plants. *Curr. Biol.* **17**, 1032–1037 (2007).
126. Meyer, S. & Scholten, S. Equivalent parental contribution to early plant zygotic development. *Curr. Biol.* **17**, 1686–1691 (2007).
127. Scholten, S., Lorz, H. & Kranz, E. Paternal mRNA and protein synthesis coincides with male chromatin decondensation in maize zygotes. *Plant J.* **32**, 221–231 (2002).
128. Xin, H. P., Zhao, J. & Sun, M. X. The maternal-to-zygotic transition in higher plants. *J. Integr. Plant. Biol.* **54**, 610–615 (2012).
129. Autran, D. *et al.* Maternal epigenetic pathways control parental contributions to *Arabidopsis* early embryogenesis. *Cell* **145**, 707–719 (2011).
130. Nodine, M. D. & Bartel, D. P. Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos. *Nature* **482**, 94–97 (2012).
131. Chen, Z. J. Genomic and epigenetic insights into the molecular bases of heterosis. *Nature Rev. Genet.* **14**, 471–482 (2013).

Acknowledgements
The authors thank B. H. Le and R. Feil for critical reading of the manuscript. F.B. and T.K. were supported by Temasek Life Sciences Laboratory. They apologize to colleagues whose publications are not cited owing to space limitations.

Competing interests statement
The authors declare no competing interests.