Chapter 2
The Role of DNA Methylation in Transposable Element Silencing and Genomic Imprinting

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Introduction

DNA methylation is the covalent modification of DNA nucleotides that may act to change chromatin structure and gene regulation. DNA methylation is a stable but reversible modification; thus, many organisms utilize this type of genomic modification for transcriptional regulation. In eukaryotes, methylation of the fifth carbon of cytosine residues is precisely maintained during mitosis and, therefore, is proposed to be involved in epigenetic regulation. Extensive studies of cytosine methylation have uncovered mechanisms for the establishment, maintenance and elimination of this modification in eukaryotes [1]. In both mammals and plants, cytosine methylation plays important roles in the silencing of TEs and genomic imprinting. In the past decade, molecular genetics and genomics approaches using mutants of *Arabidopsis* (*Arabidopsis thaliana*) have revealed many new aspects of TE silencing and genomic imprinting regulated by DNA methylation in plants. In this chapter, we describe the current models derived from these studies.

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O. Pontes, H. Jin (eds.), *Nuclear Functions in Plant Transcription, Signaling and Development,* DOI 10.1007/978-1-4939-2386-1_2

Transposable Element and DNA Methylation

TEs in Plants

Transposable elements (TEs) are mobile DNA fragments first discovered in the maize (*Zea mays*) genome by Barbara McClintock [2] that are now known to be present in nearly all eukaryotes. Some TEs contain genes whose products facilitate autonomous movement in combination with factors encoded by the host genome, whereas nonautonomous TEs lack movement genes and rely on factors encoded by other TEs. According to their mode of mobility, TEs can be divided into two main classes [3]: class I TEs, called retrotransposons, use a "copy-and-paste" mechanism in which the element replicates via reverse transcription of messenger RNAs (mRNAs), and the duplicates integrate into other chromosomal locations and class II TEs, also called DNA transposons, transpose by a "cut-and-paste" mechanism in which the elements are excised from the chromosome and integrate into new locations. A subclass of DNA transposons, the *helitrons*, have a different mechanism to transpose, called the "rolling-circle" mechanism, in which a single strand of the element is nicked and invades another chromosomal location. Plant species have all classes/subclasses of TEs, although the most common ones in plant genomes are class I TEs.

High TE content and genome size are strongly correlated. In the relatively small genome (120 Mb) of *Arabidopsis*, TE fragments constitute approximately 17% of the genome [4]. In contrast, maize, with a 20-fold larger genome, 2.3 Gb, has TE fragments represent 85% of the genome [5]. TE activities can potentially change the expression and function of genes near their insertion site and can also cause chromosome breakage leading to genome rearrangement. Such changes in the genome may generate genetic variations that contribute to the adaptation and evolution of host plants [6]. Furthermore, plant genomes have developed mechanisms to silence TE activities to prevent the disruption of normal regulation and gene function that would render deleterious effects on plant growth. Mutant studies of *Arabidopsis* in which TE activities are completely silent, have demonstrated that several layers of epigenetic regulation play a major role in repressing TE activities [7]. Notably, the role of one of the layers in TE silencing, DNA methylation, has been investigated extensively. Because DNA methylation can be copied into a newly synthesized strand during DNA replication, the silenced status of TEs is inherited stably. In the remainder of this section, we describe how DNA methylation contributes to the regulation of TE activities.

DNA Methylation in TE Silencing

The involvement of DNA methylation in TE silencing was implicated in studies of maize class II TEs, *Mu, Ac* and *Spm* in the 1980s [8–11]. These investigators com-

Fig. 2.1 Variegated phenotype of a *met1*-derived epiRIL where mobilization of *EVD*/*COPIA93* is active. *EVD*/*COPIA93* transposed into *VAR2* gene somatically, resulting in sectored leaf variegation [21]. *met* dna methyltransferase, *epiRIL* epigenetic recombinant inbred lines, *EVD* Évadé, *VAR* YELLOW VARIEGATE. (Photograph was taken and provided by Dr. Olivier Mathieu)

pared the DNA methylation level of silent TEs with that of active TEs and found that DNAs of the former were hypermethylated and those of the latter were hypomethylated. Consistent with the first observations in maize, genome-wide analyses of DNA methylation in the past decade have demonstrated that the CG and non-CG sequence context (CHG and CHH, H is C, A, T) of silent TEs, both of class I and class II, are hypermethylated in *Arabidopsis* [12–15]. Our knowledge on the roles of DNA methylation in TE silencing has been augmented by the use of *Arabidopsis* mutants defective in DNA methylation, in which some silent TEs were transposed within their genome (Fig. 2.1). The first reported mobile TE was a member of the CACTA family of class II TE found in an inbred *ddm1* (decrease in *dna methylation1*), a mutant of a chromatin remodelling adenosine triphosphatase (ATPase) involved in both CG and non-CG methylation of heterochromatic regions [16, 17]. The CACTA family of TEs is not the only family able to transpose since some other class II TEs of the *Mutator* family are also mobile in *ddm1* inbred lines [18–20]. In more recent studies, transposition of class I TEs, the most abundant TE family in the *Arabidopsis* genome, was observed in inbred progeny of *ddm1* and *methyltransferase* 1 (*met1*), a mutant of a methyltransferase essential for the maintenance of CG methylation [20, 21].

Most TEs contain and are probably silenced by both CG and non-CG methylation [12, 14]. Indeed, expression of *ATGP3*, a class I TE in the *gypsy* family, does not occur in single mutants of *met1* or *chromomethylase3* (*cmt3*), a mutant of a methyltransferase for non-CG methylation maintenance, but does occur in the *ddm1* and *met1 cmt3* double mutants [20]. This finding suggests a redundant function of CG and non-CG methylation in the transcriptional silencing of the TE. For

another example, *EVD* (*Évadé*)/*ATCOPIA93*, a class I TE in the *copia* family, can be activated transcriptionally in the *met1* single mutant, but the double mutant of *met1* with *drm2* (*domains rearranged metyltransferase2*), a mutant of another non-CG methyltransferase, shows a synergic increase in *EVD/ATCOPIA93* transcription [21]. Interestingly, the *met1 cmt3* double mutant did not show such a synergic effect in *EVD/ATCOPIA93* transcription. CMT3 and DRM2 function as DNA methyltransferases downstream of histone methylation and siRNA activities, respectively [1]. Thus, *ATGP3* and *EVD/ATCOPIA93* are transcriptionally inactivated by CG and non-CG methylation, although the modes for non-CG methylation are different.

Selective Regulation for Mobilization of TE

In the *met1* mutant, CG methylation of TEs is eliminated resulting in their transcriptional activation, but most TEs are not transposed [12, 14]. The majority of TEs would have some mutations in their protein coding sequences allowing for post-transcriptional regulation in transposition, but some TE sequences seem to encode intact proteins. This indicates that there are other processes repressing transposition in addition to transcriptional silencing. Transposition of *EVD/ATCOPIA93* can be observed in the *met1* mutant, but not in the first generation of homozygous plants [21]. *EVD/ATCOPIA93* transposition is detected beginning with the second generation and increases as the generations progress. Such a progressive mobilization was also observed in *met1*-derived epigenetic recombinant inbred lines (epiRILs). The *met1* epiRILs are genetically identical to wild type but display mosaic DNA methylation patterns in their genomes that were generated by self-inbreeding wild-type *MET1*+/+plants selected in the F2 generation after a cross between wild-type and *met1* plants [22]. *EVD/ATCOPIA93* began to mobilize beginning with the F4 generation in an epiRIL in which their transcription is active [21]. In contrast, no mobilization of *EVD/ATCOPIA93* was observed even beyond the F9 generation in an epiRIL in which DNA methylation of this TE was retained to maintain transcriptional silencing. Mobilization requires transcription, but mobilization activity is not associated with the transcription level as described in the next paragraph. In addition to *EVD/ATCOPIA93*, class II TEs of the CACTA family can be mobilized progressively in some epiRILs, although they cannot be progressively mobilized in the parental *met1* single mutant [22]. Similarly, in inbred *ddm1* mutants, class I TEs in the *gypsy* and *copia* families including *EVD/ATCOPIA93* are transposed, and the transposition of each TE occurs stochastically and independently in the inbred lines [20]. Taken together, these observations suggest that there are additional and specific repressing processes for the transposition of each TE in contrast to their transcriptional silencing maintained ubiquitously by DNA methylation and that inbreeding releases this repression somehow.

Although the repression mechanisms are still unknown, inbreeding can be skipped for the transposition of some combinations of mutants [21]. In double mutants of *met1* and *nuclear RNA polymerase d2a* (*nrpd2a*), a mutation in the common

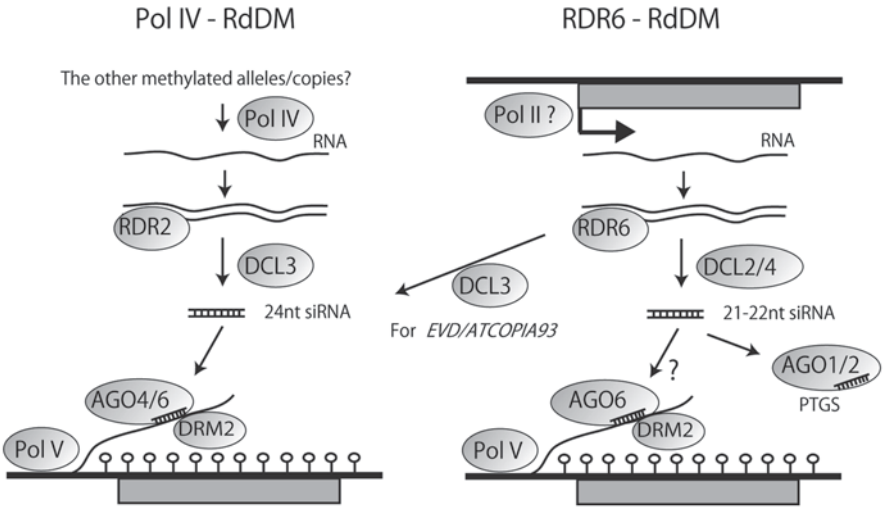

Fig. 2.2 Two pathways for the remethylation of active TEs in demethylated loci when methylation activity is restored. A 24-nt siRNA produced from the PolIV–RDR2–DCL3 pathway and a 21-nt siRNA from the RDR6–DCL2/4 pathway act on DNA methylation. In conventional RNA-directed DNA methylation (PolIV-RdDM; *left*), a 24-nt siRNA is incorporated with AGO4/6 and recruits a DRM2 *de novo* methyltransferase to a target guided by a PolV-transcribed RNA. In RDR6–RdDM, a 21–22-nt siRNA probably induces methylation of the target in cooperation with AGO6 and PolV [27] in a similar way to PolIV–RdDM. The 24-nt siRNA may be from other methylated alleles/loci homologous to the target since PolIV transcribes RNA from methylated loci. In contrast, the 21-nt siRNA would be derived from PolII-dependent RNA from a demethylated target. In *EVD/ATCOPIA93* remethylation, a 24-nt siRNA can be produced from RDR6-dependent dsRNA [28]. *TE* transposable element, *nt* nucleotide, *siRNA* small interfering RNA, *Pol* RNA polymerase, *RDR* RNA-DEPENDENT RNA POLYMERASE, *DCL* DICER-LIKE PROTEIN, *RdDM* RNA-dependent DNA methylation, *AGO* ARGONAUTE, *DRM* DOMAIN REARRANGED METHYLTRANSFERASE, *dsRNA* double-stranded RNA

subunit of plant-specific RNA polymerases, PolIV and PolV, involved in RdDM [1], as the details are described again in the next subsection (Fig. 2.2), transposition of *EVD/ATCOPIA93* was observed as well as a synergic increase in transcription. In contrast, transcription increased but TEs were not transposed in the *met1 drm2* double mutant, although DRM2 acts with PolV and siRNA produced by PolIV in RdDM. This result suggests an unknown RdDM-independent mechanism for repression of transposition. Another mutant combination showing a synergic effect for *EVD/ATCOPIA93* transposition is *met1* and *kryptonite* (*kyp*)/*suvh4*, a histone methyltransferase mutant; although a synergic increase in transcription was not observed, unlike the *met1 nrpd2a* double mutant. KYP/SUVH4 histone methyltransferase acts in the non-CG methylation pathway via CMT3 methylation activity [1]. In the *met1 cmt3* double mutant, however, there was no transposition of *EVD/ATCOPIA93*. These results suggest that the *post-transcriptional* activity of *EVD/ATCOPIA93* transposition is regulated by unknown mechanisms involving PolIV and/or PolV, and KYP/SUVH4 methyltransferase, but probably not via DNA methylation as previously proposed [21].

Resilencing Dynamics for Active TE

Transcription of subsets of TEs activated in mutants deficient in DNA methylation is repressed again when maintenance of DNA methylation is restored. A study using *ddm1*-derived epiRILs and mutants of RdDM components revealed that transcriptional silencing is achieved by re-establishment of DNA methylation by the action of RdDM [23]. In RdDM, 24-nt siRNAs produced by DICER-LIKE PROTEIN3 (DCL3) from double-stand (dsRNA) originated by Pol IV and RNA-dependent RNA POLYMERASE2 (RDR2), recruit Pol V, ARGONAUTE4 (AGO4), and in some instances AGO6, to guide DRM2 in the establishment of de novo DNA methylation (Fig. 2.2) [1]. In addition, components of post-transcriptional gene silencing (PTGS) that act in antiviral and antibacterial defences are suggested to function in DNA methylation to silence other TEs. In contrast to RdDM, a 21–22-nt siRNA produced by RDR6, DCL2 and DCL4 is associated with AGO1 and AGO2 to degrade target RNAs in PTGS [24]. A 21–22-nt siRNA produced by PTGS components, not a 24-nt siRNA in conventional RdDM, is required for transcriptional silencing in the *helitron* and *copia* elements [25, 26]. Genome-wide analyses for siRNAs in the *rdr6* and *ddm1* mutants revealed that RDR6-dependent 21–22-nt siRNAs triggered the resilencing of subsets of TEs activated in the *ddm1* mutant [27]. Thus, resilencing of TEs can be accomplished by two kinds of siRNAs produced from dsRNAs synthesized with PolIV–RDR2 and those with RDR6 (Fig. 2.2). Once DNA methylation is established, TE silencing is maintained by MET1 for CG methylation and the conventional RdDM for the others.

In contrast, other TEs cannot be remethylated even after the restoration of DNA methylation activity, and, in some cases, TEs like *EVD/ATCOPIA93* begin to transpose within the genome [21, 28]. Host plants have a mechanism to repress the activity of mobile TEs preventing an excess invasion of the genome. In *met1*- and *ddm1*-derived epiRILs, *EVD/ATCOPIA93* copy number increases with proceeding generations, but this increase stops at approximately 40 copies per genome [28]. This peak copy number seems to be fixed because several lines of both *met1* and *ddm1*-derived epiRILs attain the same copy number, whereas the number of generations required to reach the peak vary among the lines. During the process, there are several steps for silencing *EVD/ATCOPIA93* [28]. First, PTGS is activated against *EVD/ATCOPIA93* expression in which 21–22-nt siRNAs for the coding region are produced, but the RNA of *EVD/ATCOPIA93* is resistant to PTGS, thereby keeping their increased copy number. When approximately 40 copies are reached, 24-nt siRNA produced by DCL3 from RDR6-dependent dsRNA induces RdDM in the coding region (Fig. 2.2), and 24-nt siRNA and DNA methylation are spread into the promoter region. Eventually, *EVD/ATCOPIA93* is silenced again at around 40 copies [28].

Reinforcement of TE Silencing during Gametogenesis

DNA methylation is important for transposon silencing; however, DNA methylation status is not always stably maintained during the life cycle. In mammals, where

DNA methylation is primarily in the CG context, methylation is erased and re-established in the germ line. Thus, DNA methylation is reprogrammed for each generation [29]. In contrast, the reprogramming of DNA methylation in sperm and egg cells has not been reported in plants, except that only CHH methylation of transposons was partly erased in sperm cells [30, 31]. Genome-wide DNA demethylation was observed in a limited plant germ cells that are not of embryonic origin. One such example is the vegetative nucleus of pollen, which is not fertilized but controls pollen germination and pollen tube elongation [32]. DNA demethylation in the vegetative nucleus is accompanied by siRNA production from transcriptionally active TEs [30, 31, 33]. The siRNA originating from the vegetative nucleus is proposed to be transported into the sperm cells where it is involved in the silencing of TEs by the *de novo* RdDM pathway [30, 31, 33]. DNA demethylation also occurs in the central cell, which is the origin of endosperm, in the female gametophyte [31]. Although the DNA methylation pattern of plant egg cells has not been investigated, siRNAs from demethylated TEs in the central cell are predicted to reinforce DNA methylation of TEs in the egg cell by a mode of action similar to the sperm cell. Indeed, the small RNAs expressed by the central cell can affect transcription of the target in the egg cell [31]. Mechanisms of demethylation in the plant companion cell in the female gamete, the central cell, are common to those for regulating genomic imprinting as described in the next section. Thus, TE silencing in plants can be reinforced at each generation.

Genomic Imprinting and DNA Methylation

Molecular Mechanism of Genomic Imprinting

Genomic imprinting occurs in mammals and plants and results from differential gene expression caused by differences in the epigenetic status of parental genomes, including asymmetric DNA methylation. In plants, genomic imprinting primarily occurs in the endosperm and controls seed development. The endosperm is the embryo-nourishing tissue whose genetic information is not directly inherited by the offspring. Many endosperm-imprinted genes have been identified in plants (Table 2.1). Plant genomic imprinting mechanisms have been mostly elucidated by genetic approaches using *Arabidopsis* mutants. Endosperm imprinting is regulated by several epigenetic pathways involving DNA methylation and histone modifications. *FWA* is known as a maternally imprinted gene regulated by DNA methylation [34]. The 5′ region of *FWA* contains two tandem repeats, and this region is highly methylated in adult tissues. In contrast, the maternal *FWA* allele in the endosperm is hypomethylated and expressed (Fig. 2.3) [35]. This asymmetric DNA methylation status between the maternal and paternal genomes is established in the central cells before fertilization. In the central cell, genome-wide DNA demethylation was proposed to occur by both passive and active mechanisms [36, 37]. In somatic tissues, CG methylation was maintained by the MET1 methyltransferase, whereas

Table 2.1 Examples of the endosperm-imprinted genes

Gene	Species	Protein family	Expression	Regulation	Reference for imprinting regulation
MEA	*Arabidopsis*	PcG protein E(z)	Maternal	DNAme, H3K27me3	[39, 40, 51]
Mez1	Maize	PcG protein E(z)	Maternal	DNAme, H3K27me3	[79, 80]
FIS2	*Arabidopsis*	PcG protein Su(z)12	Maternal	DNAme	[38]
Fie1	Maize	PcG protein Esc	Maternal	DNAme, H3K27me3	[80, 81]
OsFIE1	Rice	PcG protein Esc	Maternal	DNAme	[82]
FWA	*Arabidopsis*	HD-ZIP	Maternal	DNAme	[34]
PHE1	*Arabidopsis*	Type I MADS box	Paternal	DNAme, H3K27me3	[48]
Mee1	Maize	Hypothetical protein	Maternal in the endosperm and in the early embryo	DNAme	[56]
SDC	*Arabidopsis*	F-box	Maternal	RdDM	[62]
MOP9.5	*Arabidopsis*	Phosphatidylinositol kinase	Maternal	RdDM	[62]

during the development of reproductive cells, *MET1* gene expression was abolished in the central cell depending on the complex consisting of RETINOBLASTOMA RELATED 1 (RBR1) and MULTICOPY SUPPRESSOR OF IRA1 (MSI) [38]. This finding suggested that passive demethylation happened in the central cell. Furthermore, DEMETER (DME), a cytosine demethylase, is expressed and contributes to active DNA demethylation in the central cell [39]. In *dme* endosperm, the DNA methylation level is higher than in wild-type endosperm [36, 37, 40]. Further analysis using single-nucleotide polymorphisms (SNPs) between different *Arabidopsis* accessions revealed the precise DNA methylation pattern of maternal and paternal genomes in the wild-type and *dme* endosperms [31]. DME seems to target TEs that are small, AT-rich and enriched in euchromatic regions. *DME* encodes a DNA glycosylase protein that is involved in the base excision repair (BER) pathway. REPRESSOR OF SILENCING1 (ROS1), DEMETER LIKE2 (DML2) and DML3 are also cytosine demethylases that are in the same family as DME but are not expressed during the reproductive phase, and their triple mutant does not show any phenotype affecting genomic imprinting. Therefore, DNA demethylation in the central cell seems to be catalyzed by DME. In *Arabidopsis*, AtLIG1 (*Arabidopsis thaliana* DNA LIGASE1) has been also reported to affect imprinting involved in the BER pathway [41]. Recently, DNA 3′ phosphatase AtZDP (*A.*

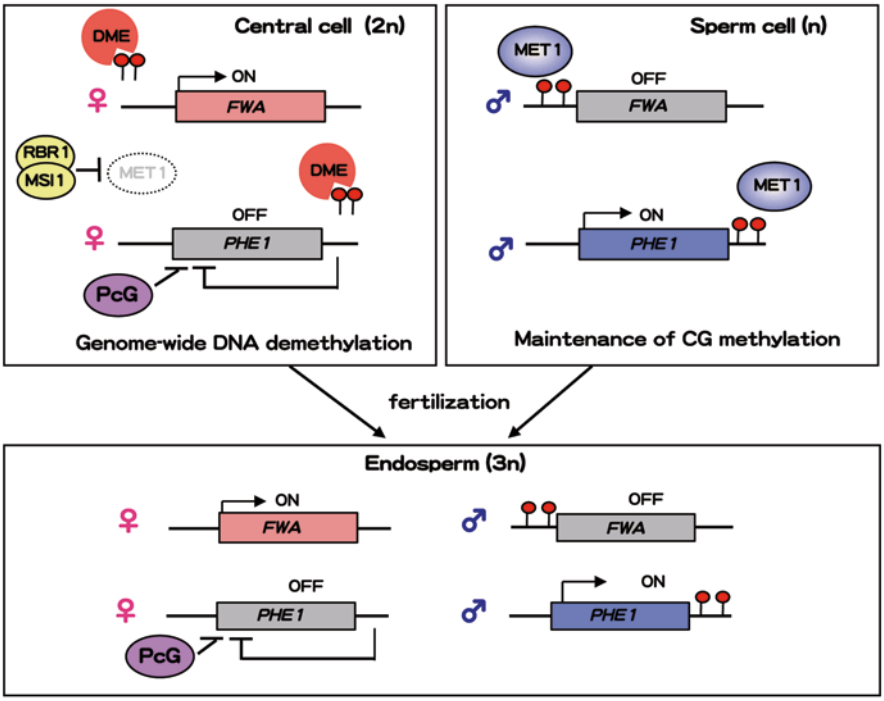

Fig. 2.3 Control of maternally imprinted *FWA* and paternally imprinted *PHE1* in *Arabidopsis*. In the central cell, genome-wide demethylation occurs by *MET1* inactivation and *DME* activation. Loss of DNA methylation in the *FWA* promoter region induces the transcriptional activation of *FWA*, whereas that in the 3′ region of *PHE1* causes PRC2-dependent H3K27me3 resulting in the *PHE1* silencing. In the sperm cell, *MET1* activity maintains CG methylation, resulting in the *FWA* silencing and the *PHE1* activation. The expression status of each allele is maintained in the endosperm after the fertilization. *PHE* PHERES, *DME* DEMETER, *PRC* polycomb repressive complex, *H3K27me3* tri-methylation at the 27th lysine of histone H3, *CG* cytosine-guanine sequence context

thaliana ZINC FINGER DNA 3′ PHOSPHOESTERASE) and DNA repair protein XRCC1 (X-RAY CROSS-COMPLEMENTING GROUP PROTEIN1) were shown to affect DNA demethylation [42, 43]. These proteins bind to ROS1 in vitro and act downstream of ROS1 involved in BER; however, it is not yet known whether they also act with DME.

MEA (*MEDEA*) is the first identified imprinted gene in *Arabidopsis* and is well characterized as a maternally imprinted gene (Table 2.1) [44, 45]. Endosperm of the *mea* mutant shows an over proliferation phenotype. Maternal *MEA* expression is controlled by DME and MET1 antagonistically [46]. However, a 200-bp minimum *MEA* imprinting control region (ICR) was recently identified, and this region was independent of DNA methylation control [47]. This result suggests an unknown regulatory mechanism for the imprinted expression of the minimum *MEA* ICR *cis*-regulatory region.

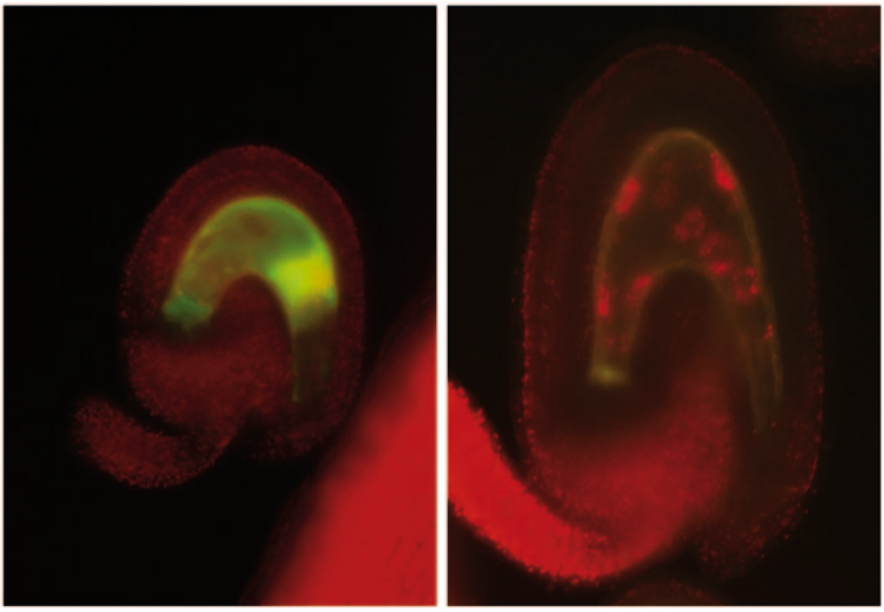

Fig. 2.4 FWA–GFP fluorescence in wild-type (*left*) and *ssrp1–3* (*right*) *Arabidopsis* ovules observed 5 days after emasculation. Fluorescence was detected in the nucleus of the wild-type central cell. FWA–GFP in the *ssrp1–3* ovule was not activated and dividing nuclei were observed without fertilization in the *ssrp1–3* ovule. Accompanying autonomous central cell proliferation, *ssrp1–3* ovules become slightly expanded. *GFP* green fluorescent protein, *ssrp* structure-specific recognition protein

Not only DNA methylation, but also histone methylation (tri-methylation at the 27th lysine of histone H3; H3K27me3) also contributes to genomic imprinting. A paternally expressed imprinted gene, *PHE1* (*PHERES1*), has a DNA-methylated region located in the 3′ region of the gene, and hypomethylation of this region is necessary for silencing via H3K27me3 by polycomb repressive complex 2 (PRC2) [48]. The hypomethylated status of the 3′ region of maternal *PHE1* makes it possible for PRC2 to access the gene and be silenced by H3K27me3 (Fig. 2.3). Genome-wide H3K27me3 profiling revealed many H3K27me3 targets like *PHE1* in the endosperm, and hypomethylation of the target region seems to be a trigger for H3K27me3 by PRC2 [49]. Interestingly, components of PRC2, FERTILIZATION-INDEPENDENT SEED2 (FIS2) and MEA are maternally imprinted, and the paternal *MEA* allele is also silenced by PRC2 in the endosperm [40, 50, 51].

Recently, additional evidence for the controlling mechanism of genomic imprinting was reported; STRUCTURE-SPECIFIC RECOGNITION PROTEIN1 (*SSRP1*) was identified as being required for the activation of maternally imprinted genes and the mutant showed an autonomous endosperm development phenotype (Fig. 2.4) [52]. In the *ssrp1* mutant, the DNA methylation level in the endosperm is higher than in the wild-type plant. *SSRP1* encodes a component of facilitating chromatin-mediated transcription (FACT), an H2A/H2B histone chaperone. FACT

controls chromatin structure during transcription and DNA replication in mammals and yeast. SSRP1 may control imprinted expression with DME by a chromatin-based mechanism. Histone H1 was identified by yeast two-hybrid screening to be a protein that interacts with DME [53]. The triple mutant of *Arabidopsis* histone H1 homologs exhibited deficiencies in DNA demethylation and expression of maternally imprinted genes in the endosperm like *ssrp1* and *dme*. Histone H1 binds to the linker region between nucleosomes and is related to chromatin structure through nucleosome compaction. These reports suggest that DNA demethylation by DME cytosine demethylase are affected by chromatin structure. Histone acetyltransferase of INCREASED DNA METHYLATION1 (IDM1) and the small RNA-binding protein REPRESSOR OF SILENCING3 (ROS3) were reported to affect DNA demethylation upstream of another demethylase, ROS1 [54, 55]. Thus, histone acetylation and small RNA-guiding mechanisms might also be related to DNA demethylation by DME.

Resetting Mechanism in the Embryo by RdDM

Unlike mammals, genomic imprinting in plants is not observed in the fully developed embryo and adult tissues. The maize *MEE1* (*MATERNALLY EXPRESSED IN EMBRYO1*) gene shows maternally imprinted expression in the early embryo and in the endosperm [56]. The maternal *MEE1* allele is hypomethylated in the early embryo and endosperm, but remethylation occurs during embryo development, and expression disappears in the mature embryo. Imprinted genes in rice embryos were reported [57]; however, imprinted expression of these genes was not detected in adult plants. In *Arabidopsis*, genome-wide evaluation of the parental genome's contribution to the early embryo (~32 cells) revealed some parental-origin specifically expressed genes, but these genes are not among the allele-specific expressed genes in the fully developed embryo [58–60]. As mentioned in the former section, Calarco et al. showed a decrease in the CHH methylation level of TEs in sperm cells and suggested that restoration of DNA methylation in the embryo depended on siRNA [30]. Indeed, the expression of DRM2 *de novo* methyltransferase was detected in the egg cell and the early embryo, and recovery of CHH methylation was observed depending on the stage of embryo development [61]. Consistent with maize *MEE1*, the resetting mechanism of DNA methylation exists in the early embryo, thus imprinted expression in the plant's adult phase may not be found.

Moreover, Vu et al. found several maternally imprinted genes controlled by RdDM. In the mutants involved in the RdDM pathway, the *drm1 drm2* double mutant and *nrpd2a*, maternal imprinted genes, *SDC* (*SUPPRESSOR OF DRM1 DRM2 CMT3*) and *MOP9.5* were mis-expressed by both paternal and maternal alleles [62]. Paternal *SDC* and *MOP9.5* may also be silenced by RdDM depending on the siRNA produced in the vegetative cells and endosperm.

DNA demethylation caused siRNA production in the central cell and in the endosperm. Indeed, maternal 24-nt siRNA accumulated in *Arabidopsis* endosperm [63]. Furthermore, in crosses between different ploidy levels of *Arabidopsis*, a maternal

siRNA level was altered and affected expression of specific AGL *(AGAMOUS-LIKE)* genes that are targets of interploidy crosses [64]. A model was proposed in which siRNA produced in the central cell and endosperm are transported to the egg and embryo, respectively. This proposal provides an explanation for why genomic imprinting is observed in the endosperm but not in the embryo.

Divergence of Imprinted Genes and Evolutionary Aspects

Genome-wide information about the transcriptome and methylome of the endosperm of several plant species is updated frequently. As summarized in the reviews [65, 66], many candidates for imprinted genes have been identified in *Arabidopsis*, rice and maize [37, 57, 58, 67–69]. To identify maternally and paternally expressed alleles, different accessions or inbred lines are used to detect SNPs between the alleles. In *Arabidopsis*, many nuclear proteins, such as transcription factors and chromatin-related proteins, and hormone signal transduction proteins were imprinted. Interestingly, components of PRC2 are commonly maternally imprinted in some plant species. These maternally imprinted genes may have positively evolved to control endosperm development.

Very few imprinted genes are common in *Arabidopsis*, rice and maize [57, 67], implying rapid evolution of imprinted genes in the plant genome. Positive selection of the *MEA* gene [70–72] and a comparison of the ratio of non-synonymous to synonymous substitutions between different species show evidence of rapid change and positive selection of imprinted genes [67, 68]. Moreover, Wolff et al. also suggested that gene duplication affects the evolution of imprinted genes [68]. For the type I MADS-box gene family, including the paternally imprinted gene *PHE1*, gene duplication and imprinted expression have a positive relationship [73]. Not only gene duplication but also TE insertion and subsequent silencing were proposed to be the driving force for imprinted expression. This theory can apply for imprinted genes regulated by DNA methylation. A theory for the evolution of imprinted genes is still controversial, but genome-wide information makes it possible to compare many imprinted genes among many species. As a result, a comprehensive view about the evolution of imprinted genes may be forthcoming.

Perspective

Genetic and genome-wide analyses using *Arabidopsis* mutants provide many new insights into the role of DNA methylation in TE silencing and genomic imprinting. However, there remain many unsolved issues in these research fields. DNA methylation acts on silencing TE at the transcriptional level, but other layers mediated by unknown mechanisms act to repress transposition. The mechanism for DNA remethylation of activated TEs has just begun to be understood. Uncovering

activation and repression systems for TEs by the host genome would lead to an understanding of the function of TEs in the genome from an evolutionary point of view. We still cannot elucidate the clear biological and evolutionary significance of genomic imprinting. Dissecting the function of newly identified imprinted genes in seed development would provide some answers for this unresolved issue.

We have discussed about the action of DNA methylation in TE silencing and genomic imprinting. However the functions of DNA methylation in plant development and environmental responses have not been identified so much compared with those in TE silencing and genomic imprinting. In mammals, DNA methylation plays pivotal roles in several developmental steps and responses to the environment [74]. DNA methylation in plants may be dynamically regulated depending on the developmental stages, specific tissues, circumstances, and so on. Plants deficient in DNA methylation in their genomes, like epiRILs, show quantitative phenotypes for development, indicating some unknown functions of DNA methylation during plant development [22, 75]. In addition, several reports describe the role of DNA methylation in several steps of plant development [76, 77]. Quantitative and comprehensive analyses with high-resolution patterns of genome-wide DNA methylation enable DNA methylation dynamics during biotic stress to be visible [78]. Thus, rapid technological progress in genome-wide analyses of DNA methylation will reveal more roles for DNA methylation than we currently know. Molecular action of DNA methylation revealed from studies of TE silencing and genomic imprinting will likely be applied to understanding the function of DNA methylation in plant development and environmental responses.

Acknowledgments We thank Dr. Olivier Mathieu (Centre National de la Recherche Scientifique, France) for his kind permission to use the photograph shown in Fig. 2.1 and Dr. Tatsuo Kanno (The National Institute of Agrobiological Sciences, Japan) for critical reading of the manuscript. Preparation of this manuscript was supported by the Programme to Disseminate Tenure Tracking System, MEXT, Japan (for YI) and Japanese Science and Technology Agency, PRESTO (for TN).

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