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MINI-REVIEW

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DNA methylation and epigenetic inheritance during plant gametogenesis

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Abstract In plants, newly acquired epigenetic states of transcriptional gene activity are readily transmitted to the progeny. This is in contrast to mammals, where only rare cases of transgenerational inheritance of new epigenetic traits have been reported (FASEB J 12:949–957, 1998; Nat Genet 23:314–318, 1999; Proc Natl Acad Sci U S A 100: 2538–2543, 2003). Epigenetic inheritance in plants seems to rely on cytosine methylation maintained through meiosis and postmeiotic mitoses, giving rise to gametophytes. In particular, maintenance of CpG methylation (^{m}CpG) appears to play a central role, guiding the distribution of other epigenetic signals such as histone H3 methylation and non-CpG DNA methylation. The evolutionarily conserved DNA methyltransferase MET1 is responsible for copying ^{m}CpG patterns through DNA replication in the gametophytic phase. The importance of gametophytic MET1 activity is illustrated by the phenotypes of *met1* mutants that are severely compromised in the accuracy of epigenetic inheritance during gametogenesis. This includes elimination of imprinting at paternally silent loci such as *FWA* or *MEDEA* (*MEA*). The importance of DNA methylation in gametophytic imprinting has been reinforced by the discovery of *DEMETER* (*DME*), encoding putative DNA glycosylase involved in the removal of ^{m}C. *DME* opposes transcriptional silencing associated with imprinting activities of the *MEA/FIE polycomb* group complex.

Introduction

There is no early deposition of germ line in plants, and gametes are formed late in development by differentiation from somatic cells in flowers. Thus, epigenetic information must be transmitted over many rounds of mitotic DNA replication in diploid sporophytic tissues, through the differentiation of gametophyte precursor cells, meiosis, and postmeiotic mitoses of haploid gametophytes. In this brief review, we focus on meiotic and gametophytic transmission of epigenetic information in plants and discuss the results of selected experiments pointing toward the roles of various components in the maintenance of epigenetic memory during the gametophytic phase of plant development.

To achieve double fertilization characteristic of seed plants, the development of male and female gametophytes requires two and three postmeiotic mitotic divisions, respectively. Male gametogenesis is initiated with the differentiation and meiotic division of diploid microspore mother cells, which originate from archespores of another primordia (McCormick 2004; Wilson and Yang 2004). After meiosis, four haploid microspores form a tetrad that later disperses free microspores (Fig. 1). The microspores undergo asymmetric mitoses, giving rise to an immature pollen grain with generative and vegetative cells. The vegetative cell ceases division, whereas the generative cell undergoes additional mitosis, leading to two sperm cells required for double fertilization (Fig. 1).

The female gametophyte differentiates from diploid ovule cells by differentiation of a megaspore mother cell, which undergoes meiotic division into four megaspores (Wilson and Yang 2004). More than 70% of flowering plants, including the model organisms *Arabidopsis* and rice, exhibit the polygonum type of gametophyte development in which three of four megaspores degenerate, leaving a single meiotic product as the functional haploid megaspore (Yadegari and Drews 2004) (Fig. 1). The megaspore undergoes three mitotic divisions to produce eight nuclei of the embryo sack: one of the egg cell, two of the synergid cells, three of the antipodal cells, and two polar nuclei that will undergo a fusion to form the diploid nucleus of the central cell (Fig. 1). In the double-fertilization process, one haploid sperm nucleus fuses with the egg cell nucleus, and the zygote develops to a diploid embryo. The other sperm nucleus fuses with the diploid nucleus of the

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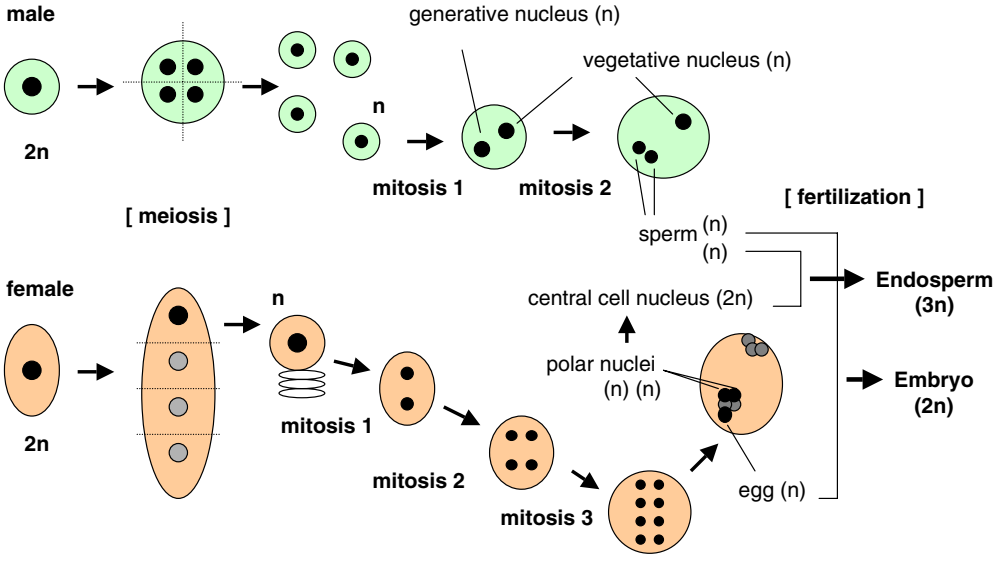

Fig. 1 Schematic representation of male and female gametogeneses in *Arabidopsis*. Two postmeiotic mitoses give rise to male gametes (sperms), whereas three rounds of postmeiotic division are required for the formation of the embryo sack

central cell to initiate the development of a triploid endosperm. Therefore, endosperm nuclei contain two maternal chromosome sets and one paternal chromosome set (2m:1p ratio). It has been documented that the 2m:1p ratio is crucial for the proper development of the endosperm (Scott et al. 1998), suggesting that the epigenetic makeup of the maternal and paternal genomes differs.

Although the proportion of loci that are epigenetically controlled in a parent-of-origin-specific manner is unknown and the general magnitude of epigenetic regulation is still difficult to estimate, it is well-established that transposable elements (and their remnants), repetitive sequences, and imprinted genes are subjected to chromatin-mediated epigenetic suppression of transcription (Martienssen and Colot 2001). Transposable elements and repetitive sequences are constituents of heterochromatin, which is mainly silent at the transcriptional level (SanMiguel et al. 1996; Arabidopsis Sequencing Consortium 2000; Fransz et al. 2000; Lippman et al. 2004). Heterochromatin and gene silencing are associated with elevated levels of 5-methylcytosine (^{m}C) and hypoacetylated histones decorated with heterochromatin-specific modifications, such as dimethylation of histone H3 at lysine 9 (H3K9me2) (Gendrel et al. 2002; Johnson et al. 2002; Soppe et al. 2002; Fransz et al. 2003; Lippman et al. 2004) or monomethylation and dimethylation of H3 at lysine 27 (H3K27me and H3K27me2, respectively) (Lindroth et al. 2004; Mathieu et al. 2005). In addition to natural chromosomal targets, transgenes are also frequently subjected to heterochromatization and transcriptional gene silencing (TGS) (Vaucheret and Fagard 2001; Probst et al. 2003). Inhibition of transgenic transcription is associated with increased levels of DNA methylation and with acquisition of repressive histone modifications at promoter sequences. De novo formation of suppressive heterochromatin is usually guided by aberrant double-stranded transcripts that are processed by the machinery of post-transcriptional gene silencing (PTGS) (Sijen and Kooter 2000), which is related to the RNA interference (RNAi) mechanism subsequently discovered in animals. TGS resulting from de novo heterochromatization may be heritable over many generations.

Here we provide a brief overview of the interplay between DNA methylation and histone modification in the initiation and maintenance of TGS, and discuss their functions in the maintenance of epigenetic information during plant gametogenesis for genes expressed biallelically and for imprinted genes exhibiting monoallelic, parent-of-origin-dependent expression.

DNA methylation and other epigenetic marks

Plants have a rather complex system of DNA methylation. In addition to a symmetrical cytosine methylation at CpG sites, as in mammals, cytosines in the CpNpG and CpNpN sequence context (where N = A, C, or T) are targets for methylation in plants (for recent review, see Tariq and Paszkowski 2004).

The first two mutations causing global reduction of ^{m}C levels were isolated in a brute-force screening for trans-acting mutations, leading to demethylation at usually hypermethylated centromeric repetitive sequences (Vongs et al. 1993). These were named *ddm1* and *ddm2* (for *d*ecrease in *D*NA *m*ethylation). In *ddm1*, levels of DNA methylation are reduced to approximately 30% through reduction of methylation at cytosines at all sequence contexts (Vongs et al. 1993). *DDM1* encodes a SWI2/SNF2-like chromatin remodeling factor (Jeddeloh et al. 1999; Brzeski and Jerzmanowski 2003). *ddm1* mutation is recessive and, in the course of inbreeding of the homozygous state, causes gradual depletion of ^{m}C. In early *ddm1/ddm1* generations, repetitive sequences, such as heterochromatin-associated centromeric and pericentromeric repeats, and transposons are preferentially affected. Several of the transposons become transcriptionally active (Jeddeloh et al. 1998; Mittelsten Scheid et al. 1998; Morel et al. 2000; Steimer et al. 2000;

Soppe et al. 2000) or even undergo transposition (Hirochika et al. 2000; Miura et al. 2001; Singer et al. 2001). *DDM1* function is crucial for the maintenance of compaction at heterochromatic centromeric and pericentromeric chromosomal regions. In *ddm1/ddm1* mutants, there is a significant decondensation of DNA at these regions in all chromosomes (Mittelsten Scheid et al. 2002; Soppe et al. 2002; Fransz et al. 2003; Probst et al. 2003). This is accompanied by a gradual replacement of heterochromatin-specific histone modifications (H3K9me2) by euchromatic specific marks (H3K4me2) (Gendrel et al. 2002; Johnson et al. 2002; Soppe et al. 2002; Lippman et al. 2004).

DDM2 encodes the DNA methyltransferase MET1, a homologue of Dnmt1 that is responsible for the maintenance of CpG methylation (^{m}CpG) in mammals. The two *ddm2* mutant alleles isolated in the initial screening (Vongs et al. 1993) were renamed *met1-1* and *met1-2* (Kankel et al. 2003). Both mutations are recessive and cause a reduction in global levels of ^{m}CpG to 30–50% of wild type and a modest reduction in ^{m}CpNpG (Kankel et al. 2003). These alleles have different missense mutations in the region that encode the catalytic domain of DNA methyltransferases. They both release TGS at heterochromatic targets (Morel et al. 2000; Steimer et al. 2000); however, they are considered to be partial losses of function, as is reflected by their relatively mild phenotypes compared with the null alleles of *met1* described below.

In an independent genetic screening for mutants impaired in the maintenance of TGS, two null alleles of *met1* (*met1-3* and *met1-4*) caused by the insertion of foreign DNA were recovered (Saze et al. 2003). In these homozygous mutant *met1* alleles, cytosine methylation of CpG at a 180-bp centromeric repeat and at several other chromosomal loci was completely erased (Saze 2003; Saze et al. 2003). Although decrease in size and decondensation of centromeric areas of heterochromatin (chromocenters) were observed in *met1-1* (Soppe et al. 2002), suggesting that MET1 activity was necessary for the maintenance of heterochromatin structure, paradoxically, compaction of centromeric heterochromatin was less affected in the null *met1-3* allele (Tariq et al. 2003). It is unlikely that the discrepancy in nuclear morphology between the two studies solely reflects different alleles, since different generations of *met1* homozygous mutant plants were examined. As it has been observed already in transgenic lines expressing antisense transcripts of *MET1*, dysfunction of *MET1* also aggravates phenotypes with inbreeding (Finnegan et al. 1996), similar to that in *ddm1*; therefore, differences in nuclear morphology may as well reflect the degree of *met1* inbreeding. This further emphasizes the complexity of the relationship between the maintenance of ^{m}CpG and the heterochromatin structure.

A subtler release of TGS, compared with *ddm1* and *met1* mutants, occurs in strains mutated in the *Chromomethylase 3* (*CMT3*) gene, which encodes a chromodomain containing plant-specific DNA methyltransferase involved in the maintenance of DNA methylation outside CpG sequences (Bartee et al. 2001; Lindroth et al. 2001). A genomewide analysis showed that depletion of *CMT3* results in a preferential loss of methylation from transposons (Tompa et al. 2002). Thus, *MET1* and *CMT3* seem to be mainly responsible for the maintenance of transposon DNA methylation at CpG and non-CpG sequences, respectively.

In contrast, *DRM* (*d*omains-*r*earranged *m*ethylase) is clearly required for de novo methylation at CpGs, CpNpGs, and CpNpNs. Mutations in *DRM* genes (especially in *DRM2*, which is responsible for most of the activity) were shown to prevent the establishment, but not the maintenance, of gene silencing at *FWA* and *SUPERMAN* (*SUP*) loci (Cao and Jacobsen 2002a). Interestingly, in contrast to numerous remnants of transposons, *FWA* and *SUP* are not associated with chromocenters (Soppe et al. 2002); however, neighboring repeats and transposon sequences seem to be involved in their transcriptional suppression. *SUP* is heavily methylated in a 65-bp hairpin-forming CpT-nucleotide-rich sequence near the transcriptional start site and in the transcribed region (Jacobsen et al. 2000), while the *FWA* promoter and 5′-untranslated region encompass retroelement-derived sequences associated with DNA methylation, H3K9me2, and accumulation of siRNA (Soppe et al. 2000; Lippman et al. 2004). Evidence for the involvement of RNAi in de novo DNA methylation mediated by *DRM*s has been reported (Chan et al. 2004), advocating the possibility of RNA-directed targeting of de novo methylation to specific chromosomal positions. It is notable that *CMT3* and *DRM* have been proposed to function in a redundant fashion (Cao and Jacobsen 2002b), both probably being involved in RNA-directed DNA methylation (Cao et al. 2003; Zilberman et al. 2003).

Deficiencies in the maintenance of DNA methylation and TGS can also be a consequence of mutations in genes that encode enzymes required for the methylation reaction itself, as exemplified by the *hog1* mutant (*HOMOLOGY-DEPENDENT GENE SILIENCING1*) (Rocha et al. 2005). *HOG1* encodes the *S*-adenosyl-L-homocysteine (SAH) hydrolase that degrades SAH. SAH is a competitive inhibitor of SAM-dependent reactions, including activities of SAM-dependent DNA methyltransferases. This indirect effect of *hog1* mutation on DNA methylation has been suggested (Rocha et al. 2005).

Importantly, components involved in the active removal of ^{m}C have also been described. The *ROS1* gene encodes DNA glycosylase and acts as a suppressor of TGS (Gong et al. 2002), which is indicated to be present by promoter hypermethylation and TGS enhancement in *ros1* mutants. The ROS1 protein shows DNA nicking activity in vitro that is directed toward methylated DNA. This supports the hypothesis that ROS1 is an ^{m}C-specific glycosylase. In an independent search for modifiers of imprinting, a second putative glycosylase specific for methylated DNA has been found. This glycosylase [named DEMETER (DME1)] is required for maternal expression of an imprinted *MEDEA* gene (Choi et al 2002). Expression of DME1 is restricted to the central cell of the embryo sack, a precursor of the endosperm in which parental imprinting regulates seed development.

The functional relationship between epigenetic marks associated with heterochromatin and TGS (e.g., DNA meth-

ylation and histone modification) has been placed under intensive scrutiny (Soppe et al. 2002; Johnson et al. 2002; Tariq et al. 2003; Jackson et al. 2002; Malagnac et al. 2002; Lindroth et al. 2004; Naumann et al. 2005; Mathieu et al. 2005). Although the details of this interplay remain to be clarified, a general picture is emerging from existing experimental data.

Studies of H3K9me2 in *met1* mutants support the notion that CpG methylation is a prerequisite for the maintenance of heterochromatic H3K9me2 (Soppe et al. 2002; Tariq et al. 2003) and for the inhibition of transcription in heterochromatin associated with acetylation of histone H4 and deposition of histone H3 methylated in lysine 4 (H3K4me) (Tariq et al. 2003). Moreover, H3K27me3, which is excluded from heterochromatin in wild type, moves into selected heterochromatic loci depleted of CpG methylation in *met1* (Mathieu et al. 2005). Thus, CpG patterns maintained during DNA replication are likely to guide other epigenetic marks such as H3K9me2 or H3K27me3.

In turn, it has been suggested that H3K9me2 directs non-CpG methylation, since a histone H3K9 methyltransferase AtSUVH4 [also named KRYPONITE (KYP)] containing a SET domain seems to be required for the maintenance of CpNpG and CpNpN methylation (Jackson et al. 2002; Malagnac et al. 2002). It has been proposed that CMT3 recognizes histone H3 that is simultaneously methylated at K9 and K27 and that this acts as a signal for DNA methylation at CpNpG and CpNpN, mediated by *CMT3*, and for TGS (Lindroth et al. 2004). Recently, studies of a further SET domain protein AtSUVH2 revealed dose-dependent effects on TGS (Naumann et al. 2005). AtSUVH2 is essential for the maintenance of a complex set of heterochromatin-specific marks on histones H3 and H4, and its loss-of-function mutation causes reduction of heterochromatic histone methylation marks and release of gene silencing. In contrast, overexpression of AtSUVH2 induces formation of ectopic heterochromatin and enhancement of TGS. Suppression or enhancement of gene silencing is associated with increased or decreased levels of DNA methylation, respectively. These changes in methylation seem to be heritable in the next plant generation. The gene silencing mediated by overexpression of AtSUVH2 requires the activity of MET1, but not of CMT3 (Naumann et al. 2005).

Maintenance of epigenetic information during plant gametogenesis

To ensure transgenerational inheritance of epigenetic information in plants, the information must be maintained during meiosis, haploid postmeiotic mitosis, gametophyte differentiation, fertilization, and embryogenesis, as well as during somatic growth and differentiation involving many rounds of mitotic divisions. In addition, balanced development of the triploid endosperm requires a defined ratio of paternal and maternal genomes, clearly pointing toward the involvement of epigenetic regulation. It has long been of interest to assess the extent to which DNA methylation is responsible for the heritability of epigenetic states and how other epigenetic marks contribute to this process in plants.

Oakley et al. (1997) used a monoclonal antibody specific to ^{m}C to follow cytological changes in DNA methylation during late male gametogenesis in tobacco. They observed a drastic reduction in the overall levels of ^{m}C in pollen generative nuclei just before pollen germination. Methylation seemed to be reduced to approximately 20% of that of the vegetative nucleus. However, extreme compaction of the generative nucleus compared with that of the vegetative nucleus could interfere with antibody penetration. Unfortunately, these tobacco experiments have not been repeated, and these results have not been confirmed for other plant species.

The role of DNA methylation and histone modifications in the transmission of epigenetic information during gametogenesis can be more precisely assessed using transgenic and genetic approaches, as performed with *Arabidopsis* strains using antisense inhibition of *MET1* expression (*MET1as*) (Finnegan et al. 1996; Ronemus et al. 1996), or with mutants affected in diverse epigenetic mechanisms. The latter approach was especially informative, since it allowed assays of gametophytic and parent-of-origin effects using simple predictions and methodologies.

It was noticed already in early transgenic experiments that DNA hypomethylation in *MET1as* plants occurs predominantly at CpG sites and that this hypomethylated state is transmitted to the next generation independent of the presence of the transgene locus inhibitory to *MET1* gene expression (Finnegan et al. 1996). This suggests that de novo DNA methylation activity is very low, at least at the examined loci, which are hypermethylated in wild-type plants. Similarly, stable transmission of hypomethylation induced by the *ddm1* mutation has been well-documented (Vongs et al. 1993; Kakutani et al. 1999). In F1 heterozygotes (*DDM1/ddm1*) produced by backcrossing of the *ddm1* homozygote to wild type, ^{m}C levels are found to be at a level intermediate between that of the two parents. Using subsequent and repeated backcross lines, as well as their selfed progenies, it has been demonstrated that the hypomethylated status originating from homozygous *ddm1* mutants can be stably transmitted during meiosis, gametogenesis, and somatic mitoses also in the presence of DDM1 activity. Therefore, as in *MET1as* plants, remethylation at loci, once hypomethylated in the absence of *DDM1*, is extremely slow—or for some loci possibly even nonexistent—even in the presence of all necessary activities existing in wild-type *Arabidopsis*. These "carry-over" effects of previously occurring demethylation and the release of transcriptional repression suggest that DNA methylation provides an epigenetic mark of primary importance that cannot be easily reset to the initial pattern after its alteration. On the other hand, it is important to notice that the *ddm1* mutation is clearly recessive, suggesting that the presence of a functional *DDM1* gene is dispensable during postmeiotic mitoses of haploid gametophytes. Obviously, it is possible that the DDM1 protein is still required during gametogenesis and that it is just carried over by somatically derived megaspore and/or

microspore mother cells in amounts sufficient for epigenetic inheritance in gametogenesis.

In contrast to *ddm1*, the effect of *met1* mutations on the haploid stage of the plant life cycle is very drastic. It has been recorded that *MET1/met1-1* heterozygote individuals with hypomethylated Landsberg-erecta-specific alleles are to be found among F2 plants from a backcrossing of a partial loss-of-function allele *met1-1* (in Columbia ecotype) to a wild-type Landsberg erecta ecotype (Kankel et al. 2003). Because *met1-1*, which is classified as recessive, was not in the homozygote state within the Landsberg background, demethylation may occur during gametogenesis depleted of MET1 activity (Kankel et al. 2003).

Subsequent studies of null *met1-3* and *met1-4* alleles clearly supported this initial observation and documented the strict requirement for *MET1* gene function for the transmission of epigenetic information during both male and female gametogeneses (Saze et al. 2003). In contrast to the straightforward recessive nature of mutations for other DNA methyltransferases (CMT3, Bartee et al. 2001; DRM, Cao and Jacobsen 2002a) or chromatin modifiers, the phenotypes of which impinge on DNA methylation (DDM1, Kakutani et al. 1999; HDA6, Murfett et al. 2001; AtSUVH4/KYP, Jackson et al. 2002), both *met1-3* and *met1-4* showed definite gametophytic effects. This was reflected by CpG hypomethylation and release of TGS also in *met1/MET1* heterozygous individuals. To unequivocally demonstrate gametophytic effects in both male and female gametophytes, heterozygous *met1-3* was crossed to a transgenic strain that was wild type for *MET1* but carried a hypermethylated and transcriptionally silenced beta-glucuronidase transgene (*GUS*) (Fig. 2). F1 plants were hemizygous for the *GUS* locus and were either wild type or heterozygous for the *MET1* locus; neither genotype released the TGS of *GUS*. This ruled out the possibility of a dominant-negative effect of *met1-3* mutation or haploinsufficiency during somatic development. To further test the gametophytic effects of *met1-3* mutation, F1 plants heterozygous for *met1-3* mutation and containing a silent *GUS* gene were backcrossed to the wild type in both directions as males or as females. Since male and female gametogeneses require two and three rounds of postmeiotic mitoses, respectively (Fig. 1), it was hypothesized that passive demethylation in *met1-3* gametogenesis should result, on average, in 50% of the fully demethylated DNA molecules and in 50% of the hemimethylated DNA molecules during male gametogenesis, and in 75 and 25%, respectively, during female gametogenesis (Fig. 2). As expected, among the *MET1-3/met1-3* heterozygous progeny after reciprocal backcrossing, 75% of maternally transmitted and 42% of paternally transmitted *GUS* loci were reactivated. Since reactivation and demethylation of *GUS* were observed in the progeny heterozygous for *MET1*, it was concluded that, once DNA is demethylated during gametogenesis in the absence of

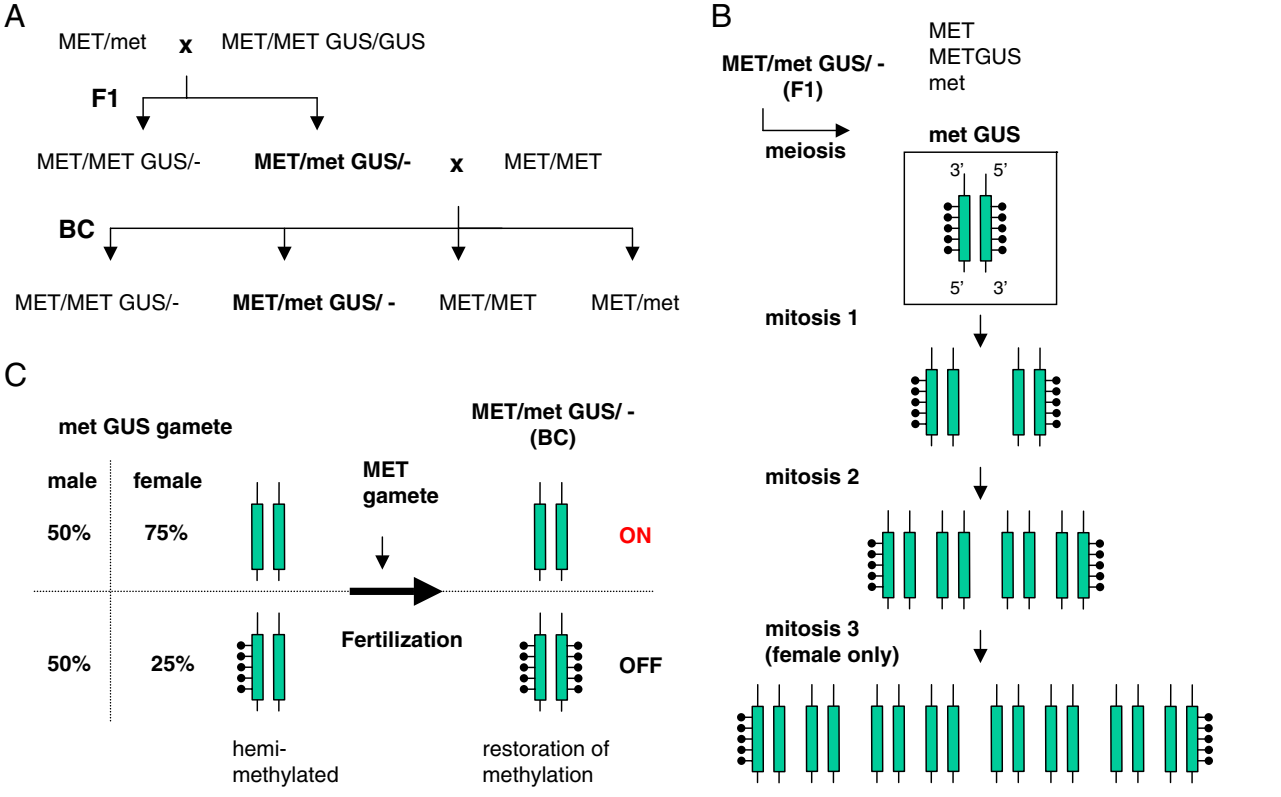

Fig. 2 Generation of demethylated epialleles during *met1-3* gametogenesis at a silent *GUS* transgenic locus. **a** Crossing scheme to test the effect of *met1-3* (*met*) on the transcriptionally silent methylated *GUS* locus after Saze et al. (2003). **b** Passive postmeiotic demethylation of the *GUS* locus during gametogenesis in a *met1-3* background. **c** Differential reactivation of maternally and paternally inherited *GUS* by *met1-3* gametogenesis. The *GUS* locus and cytosine methylation are represented by a *green box* and *black lines with lollipops*, respectively. *BC* Backcrossing

MET1 function, it cannot be readily remethylated. Thus, a methylation mark propagated by MET1 provides a blueprint for TGS and cannot be replaced easily by other epigenetic signals. Since a predicted proportion of progeny that passed *MET1*-deficient gametogenesis did not express GUS in any tissues, and since plants with chimeric GUS expression were not observed, it could be concluded also that the hemimethylated DNA molecules are likely to acquire full methylation shortly after fertilization when MET1 activity of the wild-type partner of the cross is provided. It is likely that this even occurs before the first round of zygotic DNA replication. Therefore, it can be speculated that the hemimethylated DNA generated during *MET1*-deficient gametogenesis carries all necessary information for the remethylation and reestablishment of TGS and that this all occurs independent of DNA replication. Although this hypothesis needs further testing, it can be safely concluded that epigenetic marks other than CpG methylation are not sufficient to provide the information required for the rapid recruitment of CpG methylation and reestablishment of TGS.

DNA methylation and epigenetic control of genomic imprinting in plants

Genomic imprinting, defined as parent-of-origin-specific expression of selected genes, has been associated with specific changes in DNA methylation and histone modifications. In mammals, most imprinted genes are organized in large domains residing within imprinting control regions (ICRs). During male or female gametogenesis, ICRs acquire distinctive DNA methylation marks. Paternal or maternal allele-specific methylation and transcriptional activity are maintained throughout postzygotic development, resulting in parent-of-origin-specific gene expression (Delaval and Feil 2004). In plants, parent-of-origin effects on seed development are apparent following interploidy crosses (Scott et al. 1998); however, in contrast to mammals, where many imprinted loci are characterized in detail, only a few examples of locus-specific genomic imprinting are documented for plants. Of these, imprinting of the *MEDEA* gene is the best characterized.

MEDEA (*MEA/FIS1*, *FERTILIZATION-INDEPENDENT SEED1*) is a key regulator of endosperm development; *mea/fis1*, as well as other *fis* mutations (*fis2* and *fis3/fie*), display parent-of-origin effects in which the mutation transmitted by the female, but not by the male, parent causes abnormal seed development (Ohad et al. 1996; Chaudhury et al. 1997; Grossniklaus et al. 1998). *MEA* encodes a SET domain *polycomb* group protein (Chaudhury et al. 1997; Grossniklaus et al. 1998), and it has been shown that only the maternally derived allele of *MEA/FIS1* is expressed in the female gametophyte, in the embryo, and in the endosperm after double fertilization (Kinoshita et al. 1999; Vielle-Calzada et al. 1999; Luo et al. 2000). In contrast, the paternally inherited allele is transcriptionally silenced. Importantly, seed abortion caused by a maternally transmitted *mea* mutation can be rescued by zygotic reactivation of the paternally inherited *MEA* allele by the *ddm1* mutation (Vielle-Calzada et al. 1999).

The crucial regulator of the maternal expression of *MEA* was identified in further screenings for mutants displaying parent-of-origin-specific effects (Choi et al. 2002). The component *DEMETER* (*DME*) encodes a protein with similarities to a class of monofunctional DNA glycosylases involved in base excision repair. *DME* is expressed in the central cell, and its transcription is turned off soon after fertilization. Expression of *DME* or the activity of its promoter is absent in the male gametophyte. *DME* is required for maternal expression of *MEA/FIS1*. Constitutive overexpression of *DME* results in ectopic *MEA/FIS1* expression in leaves and in the release of silencing of the paternal *MEA/FIS1* allele in the endosperm. It has been shown that the overexpression of *DME* results in nicks at the *MEA/FIS1* promoter (Choi et al. 2002, 2004). Therefore, it was proposed that expression of *DME* restricted to the female gametophyte is responsible for the regulation of imprinting by the removal of DNA methylation at the *MEA/FIS1* locus.

New mutant *met1* alleles were identified in a genetic screening for mutants suppressing *dme-1* effects on seed abortion (Xiao et al. 2003). It has been shown that *MET1* regulates *MEA* expression in the female gametophyte in a manner antagonistic to *DME* (Xiao et al. 2003). Thus, *DME* and *MET1* seem to play opposite roles in the control of genomic imprinting in plants. Interestingly, it has been suggested that MET1 cannot reverse the epigenetic modification of *MEA* following demethylation performed by *DME* after fertilization, although *MET1* is expressed in the endosperm in the absence of *DME* expression (Xiao et al. 2003). This is in agreement with the observation above that the loss of CpG methylation marks cannot be rapidly and precisely reestablished. Regarding the mechanism by which *DME* reactivates the expression of the maternal *MEA* allele, two alternatives have been put forward: (1) the excision of ^{m}C by DNA DME glycosylase, as has been implied before for mammalian DNA glycosylases (Jost et al. 2001) or *Arabidopsis* ROS1 (Gong et al. 2002), or (2) the creation of nicks in the DNA by the nicking activity of DME, which would induce local chromatin remodeling influencing the DNA methylation/reactivation of target genes (Choi et al. 2002; Xiao et al. 2003).

A similar mechanism involving antagonistic activities of *MET1* and *DME* has been proposed for the regulation of maternal endosperm-specific expression of the *FWA* gene (Kinoshita et al. 2004). In the wild type, expression of *FWA* is confined to the central cell and the endosperm. Notably, the endosperm-specific expression is correlated with loss of DNA methylation at CpGs, CpNpGs, and CpNpNs at the direct repeats within the promoter of *FWA*. As in the case of *MEA*, *DME* is required for the maternal expression of *FWA*. The paternal allele of *FWA* is silenced by DNA methylation, and this silencing is released by *met1*, but not by *cmt3* or *drm1/2*, mutations, suggesting that the removal of CpG methylation is of primary importance for silencing release.

MEA/FIS1, *FIS2*, and *FIS3/FIE* encode components of a *polycomb* group protein complex (Grossniklaus et al. 1998; Ohad et al. 1999; Luo et al. 2000; Birve et al. 2001), which also interacts with a WD-40 protein MSI1 (Köhler et al. 2003a). This complex was suggested to suppress selected maternally imprinted genes. Recently, transcriptional profiling of *mea* and *fie* mutants revealed a MADS box gene *PHERES1* (*PHE1*) as the direct target of *MEA/FIS/FIE*-mediated regulation (Köhler et al. 2003b). *PHE1* is silenced in the female gametophyte of wild-type plants but is expressed in *fis* mutants. Using chromatin immunoprecipitation assays, both MEA and FIE were shown to bind the *PHE1* promoter. Also, evidence that maternal repression of *PHE1* is mediated by maternally expressed *MEA* was provided (Köhler et al. 2005).

So far, parental imprinting in plants seems to be restricted to the endosperm; however, it has been observed in *met1-3* and *ddm1-5* mutants that a specific single-gene array of 5S rDNA becomes distinctly marked with H3K27me3. This can be seen in leaf nuclei and occurs in an apparently monoallelic manner, raising the possibility of parent-of-origin regulation at the 5S rDNA loci that may occur in somatic tissues (Mathieu et al. 2005). Further studies are required to test this hypothesis.

Conclusion

It appears that maintenance of DNA methylation patterns at CpG sites is a key process that secures epigenetic inheritance during plant gametogenesis. This process plays a fundamental role in the propagation of chromatin-structure-based regulation of gene and chromosomal activities.

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