African Journal of Biotechnology Vol. 9(50), pp. 8506-8514, 13 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1941
ISSN 1684–5315 ©2010 Academic Journals

Review

Roles of DNA methyltransferases in *Arabidopsis* development

Junjun Huang[1]*, Huahua Wang[2], Xiaojun Xie[1], Dan Zhang[1], Yan Liu[1] and Guangqin Guo[1]*

[1]Institute Of Cell Biology, School Of Life Science, Lanzhou University, Lanzhou, 730000, PR China.
[2]Institute of Plant Physiology, School Of Life science, Lanzhou University, Lanzhou, 730000, PR China.

Accepted 9 September, 2010

DNA methylation plays a vital role during development in gene expression and chromatin organization. DNA methyl transferases catalyze the transfer of a methyl group to bases within the DNA helix. Plants differ from animals in having methylation at the sites of CHG and CHH. In plant, there are at least four classes of cytosine methyltransferase: MET1, CMT3, DRM and DNMT2. They show distinct expression patterns and levels in tissues and developmental stages and differential activity on cytosines in different sequence contexts. Mutations that cause severe loss of DNA methylation often leads to abnormal development. In the present review, we summarized recent findings of the three major DNA methyltransferases mutants playing vital role in development of *Arabidopsis thaliana*.

Key words: DNA methylation, epigenetics, methyltransferase, mutant.

INTRODUCTION

DNA methylation is a major epigenetic event and is found widely in the genomes of prokaryotes and eukaryotes.

*Corresponding author. E-mail: huangjj04@lzu.cn; gqguo@lzu.edu.cn.

Abbreviations: CMT3, Chromomethyltransferases 3; **DDM1,** decrease in DNA methylation 1; **DNMT1,** DNA methyltransferase 1; **DNMT2,** DNA methyltransferase 2; **DNMT3a,** DNA methyltransferase 3a; **DNMT3b,** DNA methyltransferase 3b; **DNMT3L,** DNA methyltransferase 3L; **DME,** demeter; **DML,** demeter-like proteins; **DRM,** domains rearranged methyltransferases; **H3K9,** Histone H3 lysine 9; **H3K27,** histone H3 lysine 27; **H3K9me2,** histone H3 dimethylation at lysine 9; **H3K27me3,** trimethylated lys 27 of histone 3; **HMTase,** histone methyltransferase; **ibm1,** increase in bonsai methylation 1; **LTR,** Long terminal repeat; **m5C,** C5-methylcytosine; **MET1,** methyltransferases1; **MEA,** medea; **MTase,** methyltransferase; **PAI,** phorphosibosyl-anthranilate-isomerase; **RdDM,** RNA-directed DNA methylation; **RRD2,** RNA-dependent tRNA polymerase; **ROS1,** repressor of silencing 1; **SET,** histone methyltransferase; **SDC,** suppressor of drm1/2 cmt3; **siRNA,** small interfering RNA; **SUP,** superman; **SUVH4,** su(var)3-9 homolog 4; **TRDMT1,** tRNA aspartic acid methyltransferase 1; **TGS,** transcriptional gene silencing; **UBA,** ubiquitin associated.

DNA methylation in higher eukaryotes has two essential roles-defending the genome against transposons and regulating gene expression (Chan et al., 2005). It acts with other epigenetic modifications such as histone modification and chromatin remodeling to regulate chromatin structure and gene expression, a process called epigenetic regulation.

In plants and fungi, genomic methylation is mainly restricted to transposons, coding sequences and other repeats. In mammals, by contrast, most DNA outside regulatory regions (intergenic DNA, coding DNA and repeat elements) appears to be methylated (Weber and Schübeler, 2007). The main role of DNA methylation in plants, controls the transcription of invading and mobile DNA elements, such as transgenes, viruses, transposons and retroelements to effect on development (Kidwell and Lisch, 2001). Plant DNA methylation in patterns can be inherited over multiple generations (Kakutani, 2002); changes in DNA methylation that arise somatically during the plant life cycle have the possibility of being propagated. Active DNA demethylation, a reverse process carried out by DME/ROS/DMLs etc., is important in pruning the methylation patterns of the plant genome, and even the normally “silent” transposons are under dynamic control by both methylation and demethylation (Zhu et al., 2007). Therefore, epimutation, a change in heritable chromatin marks, might be an important source

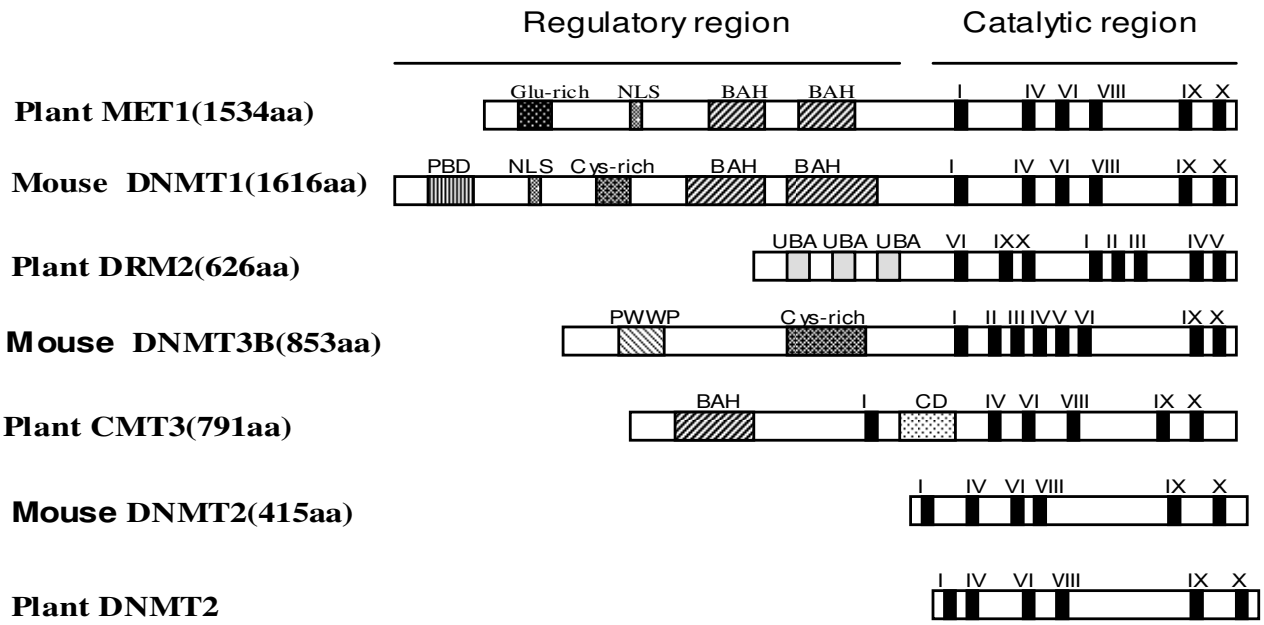

Figure 1. Schematic diagrams of plant DNA methyltransferases. There are four classes of DNA cytosine methyltransferases in *Arabidopsis thaliana* genome. The methyltransferases are divided into an N-terminal regulatory part and a C-terminal catalytic part. The size of each protein is indicated in amino acid numbers, and conserved motifs in the catalytic region are indicated by closed boxes with Roman numerals defined for the prokaryotic enzymes from the data base. Briefly, the conserved catalytic motifs I and X are involved in the AdoMet binding. Motif IV, which contains the invariant prolylcysteinyl, doublet has been identified as the functional active site of all known C5-MTases. Motif VI provides a glutamic acid that plays an important role in the target cytosine binding. Motif VIII is suggested to make nonspecific contacts with cytosine, which contribute to the neutralization of the negative charge of the DNA backbone. Motif IX is involved in the organization of the target recognition domain (TRD) (Pavlopoulou and Kossida 2007). Specific regions in the regulatory region are indicated by shaded boxes with appropriate names. Glu-rich, glutamine rich acidic region, NLS, nuclear localization signal; BAH, bromo-adjacent homology domain; PBD, PCNA binding sites; Cys-rich, cysteine-rich region; UBA, ubiquitin association domain; PWWP, Pro-Trp-Trp-Pro domain; CD, chromodomain.

of variation during plant evolution (Gehring and Henikoff, 2007).

DNA methylation occurs not only in CG dinucleotide, but also at CHG and CHH (an asymmetric site, where H is A, C or T) sequences in plants. In the *Arabidopsis* genome, the CG sites are generally methylated over 80% or not at all, whereas CHG sites are typically 20 - 100%, and CHH sites tend to be below 20% (Cokus et al., 2008). Approximately 20% of its genome is methylated, with transposons and other repeats comprising the largest fraction, whereas the promoters of endogenous genes are rarely methylated (Zhang, 2008). Usually, DNA methylation is catalyzed by various methyltransferase enzymes. These proteins modify cytosine residues specifically in definite DNA nucleotide sequences (Vanyushin, 2005). All methyltransferases utilize a common catalytic mechanism and employ AdoMet cofactor (S-adenosylmethionine) as the source of the methyl group (Eden et al., 2003; Villar-Garea et al., 2003). These functions are separated into two domains: the variable N-terminal domain and the catalytic C-terminal domain, respectively. In mammals, DNA methylation patterns are established and maintained by at least five methyltransferases enzymes: DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L (Bestor, 2000; Li, 2002). So far, the plant DNA methyltransferases have been identified and grouped into four main families based on their linear domain arrangement: methyltransferases 1 (MET1), chromomethyltransferases 3(CMT3), the domains rearra-nged methyltransferases (DRM) and DNMT2 (Figure 1). MET1 and CMT3 are presumed to be responsible for the maintenance of CG and CHG methylation, respectively, whereas DRM appear to be the principal *de novo* methyl-transferase. DNMT2, which is lacking the N-terminal domain, is conserved in many eukaryote genomes, but its function is unknown (Finnegan and Kovac, 2000; Bartee and Bender, 2001; Cao et al., 2003). In *Arabidopsis*, the methyltransferase genes are dispersed across all five chromosomes besides the chromosome II, with most of the genes occurring as families consisting of two to three members (Figure 2A). That may result from genome duplication and gene reshuffling. METs locate on chromosome IV except MET1 which may result from large-segment gene-duplication and share one ancestral gene with CMT (Figure 2B) (Pavlopoulou and Kossida, 2007). It is tempting to think that all the methyltransferase genes may share a common ancestor, incline to different polarization during evolution and carry out diverse function

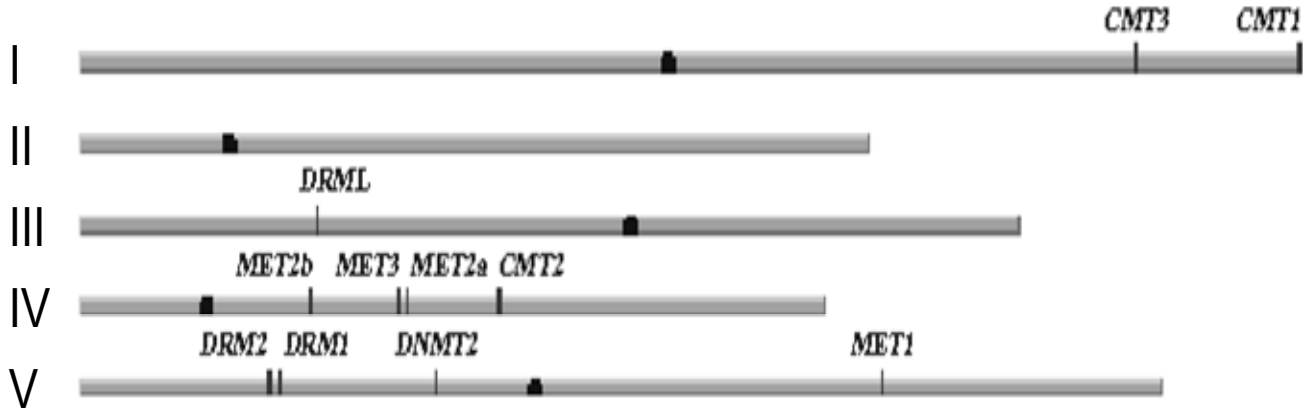

Figure 2. Genomic structure and phylogenetic relationship between the members of the *Arabidopsis* methyltransferase gene family. A, Physical map showing the distribution of the *Arabidopsis* methyltransferase genes (Table 1) among the five chromosomes with tags indicating genes (http://www.*arabidopsis*.org/servlets/sv) B, Phylogenetic analysis to infer the evolutionary relationships of DNA methyltransferases by employing the Neighbor-Joining method (Saitou and Nei, 1987). Phylogenetic analyses were conducted in MEGA 4.0.1 (Tamura et al., 2007). Mus stands for mouse.

by further duplication based on the order and orientation.

In this review we summarize the properties of the three main classes of plant DNA methyltransferase in *Arabidopsis* and their important roles in development, highlighting their emerging interactions uncovered by the most recent investigations on multiple mutants of different methyltransferase classes and other molecular components that are required for correctly methylating genomic DNA.

MET1: MAINTENANCE OF CG DNA METHYLATION

MET1 is similar to the mammalian Dnmt1. They share 50% amino acid identity in the C-terminal catalytic domain but only 24% identical in the N-terminal regulatory domain (Figure 1) (Finnegan and Dennis 1993; Finnegan and Kovac, 2000). It is encoded by a member of a multigene family with four characterized genes *(*METI*,* METIIa, METIIb, and METIII) (Table 1) that contain conserved intron positions (Genger et al., 1999). *Arabidopsis met1* mutants display severe reduction in CG methylation and moderate loss of non-CG methylation in the genome (Kankel et al., 2003), and are responsible for the release of transcriptional gene silencing and morphological abnormalities such as delayed flowering and reduced fertility (Finnegan et al.,1996; Kankel et al., 2003; Saze et al., 2003). But mutant alleles are weak with no morphological phenotype and accumulate numerous epimutations due to the loss or gain of endogenous gene silencing via following inbreeding (Jacobsen et al., 2000; Soppe et al., 2000; Kankel et al., 2003). Zilberman et al., (2007) compare global transcript levels in *met1* and wild type plants and find that the expression of both methylated and unmethylated genes increase in *met1*, with the formers more significantly. Also, a few genes have been found to be mis-expressed in met1 and are responsible for some of the developmental phenotypes. Such as ibm1 (increase in bonsai methylation 1) (Saze et al., 2008), Sadhu6-1 (a non-autonomous retroposon) (Rangwala and Richards, 2007) or RPS (a repetitive hypermethylated Petunia hybrida DNA fragment in transgenic *Arabidopsis* line RA5) (Singh et al., 2008) are reported to be affected by CG methylation in met1 background.

In the gametophytic phase MET1 is responsible for copying ^{m}CG (Figure 3A) patterns through DNA replication. In the absence of CG methylation, new and aberrant epigenetic patterns are progressively formed over several plant generations (Mathieu et al., 2007). That is illustrated by the phenotypes of *met1* mutants which are severely compromised in the accuracy of epigenetic inheritance during gametogenesis, including elimination of imprinting at paternally silent loci such as FWA or MEDEA (MEA) (Pien and Grossniklaus, 2007). Imprinting, the phenomenon of expression in a parent-of-origin-specific manner either from the paternal or maternal allele, is not established by acquisition of DNA methylation, rather it is correlated to DEMETER (DME) in endosperm of flowering plants (Arnaud and Feil 2006; Kinoshita et al., 2004). DME encodes putative DNA glycosylase and involves in the removal of ^{m}C. To date, all imprinted genes identified in plants are expressed in the endosperm and maintenance of DNA methylation is essential for parental imprinting during the *Arabidopsis* life cycle (Jullien et al., 2006). Analysis on met1 mutant also shows that maternal and paternal genomes play distinct roles in the regulation of seed size; paternal genome hypomethylation causes seed size reduction, whereas maternal genome hypomethylation has no effect on seed size (FitzGerald et al., 2008; Berger and Chaudhury, 2009). That is why MET1 is necessary for the maintenance of methylation during gametogenesis of the *Arabidopsi thaliana* life cycle (Saze et al., 2003; Xiao et al, 2003; Kinoshita et al., 2004).

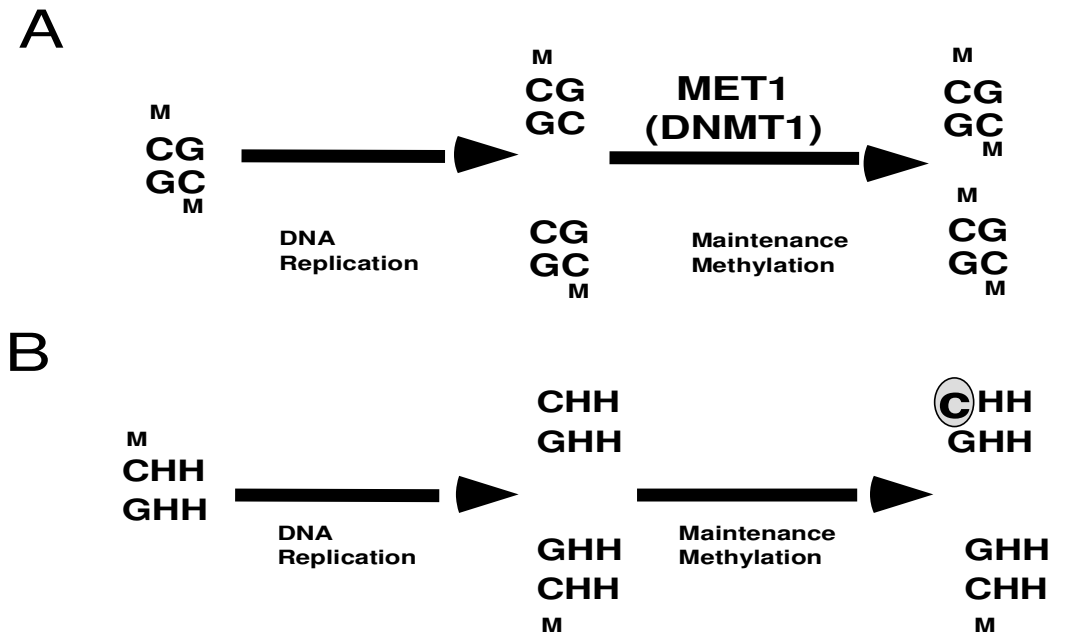

Figure 3. Cytosines are often methylated by *de novo* cytosine methyltransferase. After semiconservative DNA replication, the result of the symmetric cytosine methylation (A.) and asymmetric cytosines methylation (B.) is different.

MET1 control heterochromatin assembly at chromocenters by its influence on CG methylation and subsequent methylation of H3K9. Removal of CG methylation results in a clear loss of H3K9me2 (Figure 4) (Soppe et al., 2002; Tariq et al., 2003). At the same time H3K27me3 moves into selected heterochromatic loci depleted of CG methylation in met1, even though H3K27me3 is excluded from heterochromatin in wild type (Mathieu et al., 2005). Methylation of H3 and H4 histones is a prerequisite for DNA methylation, but not vice versa (Fuchs et al., 2006). Actually, histone modifications and chromatin remodeling are vital in the differential epigenetic control of repeats and genes. The above mentioned strongly suggest that maintenance of CG DNA methylation is critical for controlling endogenous gene expression and normal plant development.

CHG sites are particularly abundant in repetitive DNA sequences and they are also found to a significantly higher degree in the heterochromatic regions. Disruption of MET1 expression induces higher levels of short interfering RNA accumulation and affects transposons and centromeric repeats (Chen et al., 2008). It is tempting to think that MET1 appears to be necessary to maintain CHG methylation. However, when the CMTs emerged, containing the chromodomain motif which interacts with chromatin proteins and may direct CMTs to the targeted heterochromatic region (Henikoff and Comai, 1998), METs possibly narrowed their preference to CG methylation targets (Pavlopoulou and Kossida 2007). Therefore, the CMT proteins maintain CHG methylation in plants, offering an evolutionary advantage to the plants (Goll and Bestor, 2005).

CMT3: THE UNIQUE METHYLTRANSFERASE IN THE PLANT KINGDOM

CMT is a second class of methyltransferase and unique to the plant kingdom, controlling non-CG methylation (Figure 3B) (Cao and Jacobsen, 2002a). It is encoded by three genes (CMT1, CMT2 and CMT3) in *Arabidopsis* (Table 1). Mutations in CMT3 lead to global loss of both CHG and asymmetric CHH methylation at repetitive centromeric regions (with minor loss of CG methylation) and transposon reactivation. But they are morphologically normal, even after several generations of inbreeding, which are in contrast to met1 mutation (Bartee et al., 2001; Lindroth et al., 2001). It may suggest that CMT3 function is specialized for only a subset of methylated regions in the genome. As known, CHG sites are particularly abundant in repetitive DNA sequences, and they are found to a significantly higher degree in the heterochromatic regions (Pavlopoulou and Kossida, 2007). However, chromocenter formation does not depend on CHG methylation in *Arabidopsis* (Fransz et al., 2006).

CMT contains a special chromodomain amino acid motif between the conserved motifs II and IV (Figure 1), which are critical for guiding proteins to heterochromatin, suggesting a role for CMT in modifying DNA in heterochromatin (Eissenberg, 2001). They may have evolved to maintain the methylation status in the heterochromatic regions of the plant genome (Papa et al., 2001). The

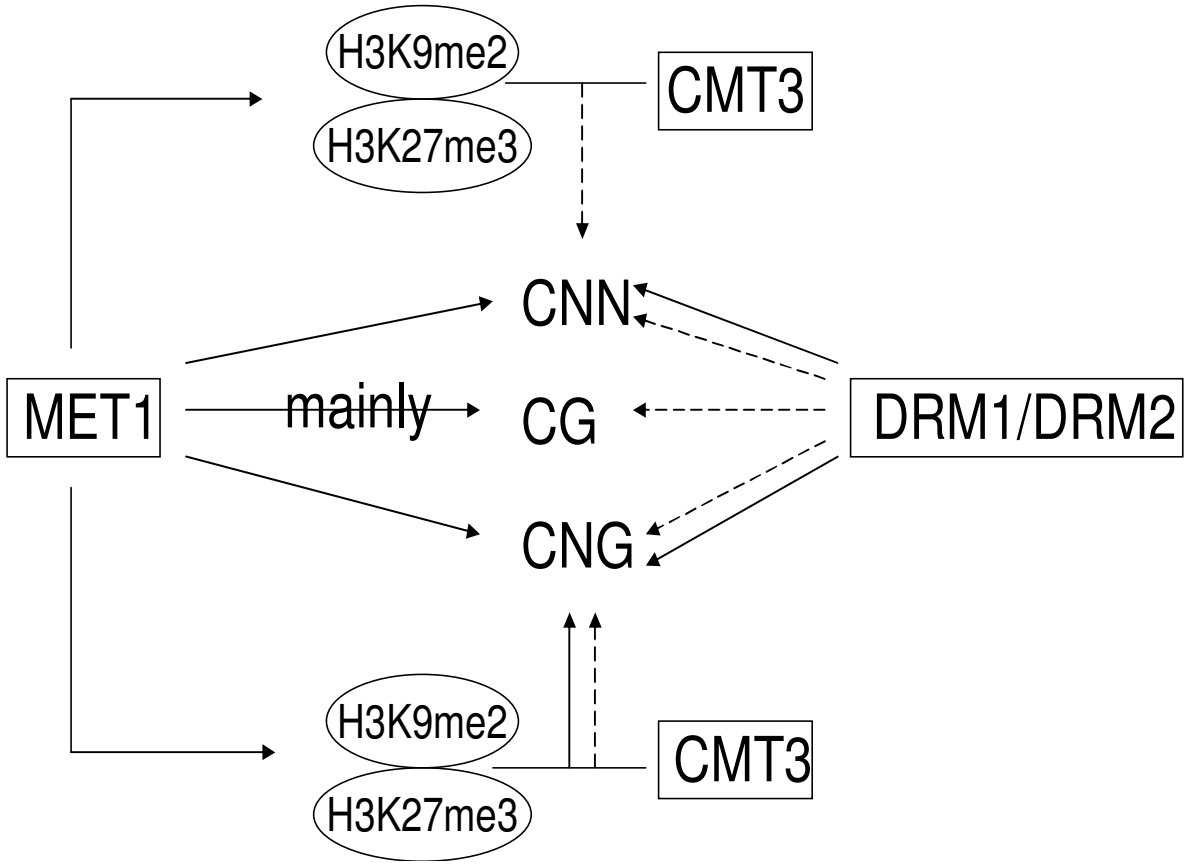

Figure 4. The network simply illustrates the genetic interactions between different methyltransferases. MET1 is essential for the maintenance of global cytosine methylation. CMT3 is involved in *de novo* methylation at non-CG positions and also maintains CHG methylation. The chromodomain of CMT3 binds histone H3 tails when positions at H3K9 and H3K27 are simultaneously methylated. CMT3, H3K9me2 and H3K27me3 are working together for non-CG methylation. DRM is responsible for virtually all *de-novo* DNA methylation and maintains non-CG methylation. Real line and broken line each stands for maintenance and establishment of methylation.

process is related to the histone H3K9 methyltransferase AtSUVH4 [also named KRYPONITE (KYP)], which contains a SET domain and seems to be required for the maintenance of CHG and CHH methylation (Jackson et al., 2002; Malagnac et al., 2002). The chromodomain of CMT3 is shown to bind histone H3 tails when the positions at H3K9 and H3K27 are simultaneously methylated (Figure 4). The above mentioned phenomenon suggests that this is a signal for DNA methylation at CHG and CHH mediated by CMT3 and for transcriptional gene silencing (TGS) (Lindroth et al., 2004).

CMT3 mutants abolish epigenetic silencing at both the SUPERMAN (SUP) and the repeat phosphoribosyl-anthranilate-isomerase (PAI) loci in *Arabidopsis* (Bartee et al., 2001; Lindroth et al., 2001). In addition to controlling CHG methylation at SUP and PAI, CMT3 also methylates several additional endogenous sequences such as the *Athila* and *Ta3* transposons (Lindroth et al., 2001). Interestingly, Sadhu6-1 with the strongest CHG methylation is expressed in a met1 CG methyltransferase mutant, but not in cmt3 mutants (Rangwala and Richards, 2007). It is interesting to determine how many genes are controlled by CMT-mediated methylation via gene-chip experiments.

DRM: THE MAJOR *DE NOVO* METHYLATION ENZYMES

DRM proteins are found only in plants. Their catalytic domains show sequence similarity to mammalian DNMT3 and possess a characteristic rearrangement in catalytic motifs, between I-V and VI-X (Figure 1) (Cao et al., 2000). However, unlike DNMT3 in mammalian, the DRMs have unique N termini containing two or three ubiquitin associated (UBA) domains that are not present in other eukaryotic methyltransferases. UBA domains show a conserved hydrophobic patch on the solvent-accessible surface (Figure 1), which may act as a common protein-interacting interface functioning in protein-protein interactions (Mueller and Feigon, 2002). The potential mechanism may be that the UBA domains recruit the DRM to

Table 1. Summary of the methyltransferases from *Arabidopsis thaliana.*

Classification	Protein name	Target sequence and function	References
METI family	AtMETI	Maintenance:CG,probably CHG; single-copy DNA ,rRNA, and centromeric repeats	Kankel et al.2003 Finnegan et al.1993
	AtMETIIa	Maintenance?	Genger et al.1999
	AtMETIIb	Maintenance?	Genger et al.1999
	AtMETIII	Maintenance?	Genger et al.1999
CMT family	AtCMT1	Nonessential	Henikoff et al.1998
	AtCMT2	Associated with heterochromatin	Genger et al.1999
	AtCMT3	Maintenance:CHG at and transposons in heterochromatin	Bartee et al. 2001 Lindroth et al. 2001
DRM family	AtDRM1	*De novo*: CG,CHG,CHH Maintenance: CHG,CHH	Cao et al.2002a Cao et al.2003
	AtDRM2	*De novo*: CG,CHG,CHH Maintenance: CHG,CHH	Cao et al.2002a Cao et al.2003
	AtDRML	Putative,unknown	
Dnmt2 family	AtDnmt2	Putative,RNA?	Goll et al.2006

certain DNA regions targeted for *de novo* methylation by interacting with chromatin proteins.

In *Arabidopsis*, there are at least three DRM related genes: DRM1, DRM2 and DRM3 (Table 1). Genetic analysis suggests that DRM1 and DRM2 may be responsible for methylation of cytosines in inverted-repeat transgenes at both CHG and CHH sites (Figure 3B). DRM1 is expressed at a much lower level than DRM2 and the drm2 mutant recapitulates all the tested phenotypes of drm1 drm2 mutants (Cao and Jacobsen, 2002b). drm1 drm2 double homozygotes show morphology that is similar to the wild-type WS strain and do not normally lose their methylation during the plant life cycle, even after five generations of inbreeding. But they block *de novo* CHG and asymmetric methylation and gene silencing of the endogenous SUPERMAN (SUP) gene. In drm2 mutant pants, genes demethylated by ROS1 accumulate methylation at CG sites (Penterman et al., 2007). This means that the DRM genes are important and required for establishing methylation in CG, CHG and CHH (Figure 4).

Interestingly, the phenotypes in mutants in RNA-silencing genes are similar to the *de novo* methyltransferase drm1 drm2 double mutant, which may indicate that the guidance of DNA methylation by siRNA. This is confirmed by a drm mutant that is found to suppress *de novo* methylation directed by generation of small interfering RNA molecules (siRNA) (Cao et al., 2003; Chan et al., 2004; Zilberman et al., 2004), although the mechanism is unknown. siRNA are preferentially associated with methylated transposable elements, but not with methylated genes (Vaughn et al., 2007). Miki and Shimamoto (2008) point out that the exogenous siRNA are strong inducers of *de novo* DNA methylation in transcribed sequences of rice endogenous genes, but are insufficient to induce heterochromatin formation. Silencing of tobacco (*Nicotiana tabacum*) LTR retrotransposon Tnt1 in *Arabidopsis* also occurs via an RNA-directed DNA methylation process, and can be partially overcome by some stresses (Pérez-Hormaeche et al., 2008).

GENETIC INTERACTIONS BETWEEN DIFFERENT METHYLTRANSFERASES AND THE BEYOND

The importance of cytosine methylation for plant development is first testified by treatment of plants with the hypomethylation agent 5-azacytidine (Richards, 1997) and later by genetic manipulations on various methyltransferase mutants showing a range of degree of reduction in total genomic cytosine methylation. Different classes of MTase appear to have their distinct target sequence texts/regions but also with some overlapping functions. In the model plant *A. thaliana*, the general scheme appears to be that MET1 is essential for the maintenance of global cytosine methylation for CG at hemimethylated sites, and may also participate in *de-novo* methylation (Aufsatz et al., 2004). CMT3 is involved in *de-novo* methylation at non-CG positions and also maintains CHG methylation. DRM is responsible for virtually all *de-novo* DNA methylation and are also required for maintenance of symmetric methylation. However, things may not be so simple: multiple mutant analysis (Zhang et al., 2006; Penterman et al., 2007; Singh et al., 2008) combined with whole genomic scale mapping of m5C at single base-pair resolution (Lister et al., 2008; Cokus et al., 2008) have revealed complicated interactions between different MTases, as well as other

cellular components/signals, such as machineries for DNA demethylation (DME, ROS and DMLs), histone modification (SUVH4), chromatin remodeling (DDM1) and those for RNA directed DNA methylation (RdDM) pathway, some of which have been described above, such as RPS. In a drm1/2/cmt3 mutant, its CG methylation is lost, and in a met1 mutant, non-CG methylation is almost completely eliminated, implying an unusual cooperative activity of all three DNA methyltransferases required for maintenance of both CG and non-CG methylation in RPS (Singh et al., 2008).

Multiple mutant analyses reveals that in the drm1/2 cmt3 triple mutant, the vast majority of non-CG methylation eliminated with little change in CG methylation, leading to several developmental defects, such as curled leaves, short stature, reduction in biomass, and partial sterility. However, single mutants are phenotypically normal even after prolonged inbreeding (Cao et al., 2003; Chan et al., 2006). SUPPRESSOR of drm1/2 cmt3 (*SDC*) is responsible for these phenotypes. It encodes an F-box protein and contains seven promoter tandem repeats, which show a unique silencing requirement for non-CG DNA methylation directed redundantly by histone methylation and siRNA (Henderson and Jacobsen, 2008). The embryos of the met1 cmt3 mutants show reduced viability concretely exhibiting incorrect patterns of cell divisions, polarity, and auxin gradients and mis-expressed genes that specify embryo cell identity. This means that DNA methylation is necessary for proper embryo development and viability in *Arabidopsis* (Xiao et al., 2006). Furthermore, met1 cmt3 adult plants exhibit a number of severe developmental defects, such as extremely late flowering, reduced leaf size, shorter stature, and complete sterility. The phenomena is the same with drm1/2 met1 mutants (Zhang and Jacobsen, 2006; Cokus et al., 2008), and met1 cmt3 double mutant is equally effective in reducing CHH methylation as is drm1/2 cmt3 (Cokus et al., 2008). drm1/2 cmt3 met1 plants can be recovered from drm1/2 cmt3 met1/+ parents and such quadruple mutant plants grow very slowly, exhibit a suite of severe developmental phenotypes, and fail to flower after about 7 months (Zhang and Jacobsen, 2006). When drm1drm2cmt3 plants are transformed with DRM2 or CMT3, the developmental phenotypes could be reversed, involving in RNA silencing, 24-nucleotide short interfering RNA (siRNA) pathway as well as histone H3K9 methylation (Chan et al., 2006). These results suggest that the methyltransferases via a net work of persistent targeting signals has been co-opted to regulate developmentally important genes. At the same time, they supported the notion that CG methylation and non-CG control important developmental genes in *Arabidopsis*. It means that methyltransferases function both independently and dependently in a complex manner to establish and maintain correct DNA methylation. It is also possible that the gross loss of DNA methylation in these multiple mutants may undermine the general structure and function of the chromosomes (e.g., chromosome segregation or heterochromatin condensation) and thus affect normal cell divisions (Zhang and Jacobsen, 2006).

Recent characterization of the DNA demethylases ROS1, DME, DML2, and DML3 in *Arabidopsis* suggests that subsets of genomic DNA methylation patterns are the products of antagonistic methylation-demethylation activity (Penterman et al., 2007). By studying rdr2 and drm*2* mutant plants, Penterman et al., 2007, find that genes demethylated by ROS1 accumulate CG methylation. They propose that this hypermethylation is due to the ROS1 down-regulation that occurs in these mutant backgrounds, suggesting an interesting interaction between ROS and DRM2/RdDM pathway. It remains to be determined how DNA demethylase activity is regulated and a precise understanding of the genomic targets of methylation and demethylation is essential to deconvolute how these opposed activities forge the methylation landscape that is observed.

In summary, so far the experimental evidences indicate that different DNA methyltransferases not only have their own roles in the establishment and maintenance of DNA methylation, but also function redundantly or interdependently in complex ways. To function properly, they also require the participant of other molecular components within the cell. Further studies should focus on how the methyltransferases interact with histone methyltransferases and chromatin-remodeling proteins to achieve DNA methylation for the regulation of gene and chromosomal activities and plant development. Future experiments on DNA methylation in different organisms may get results beyond our imagination. So far the most of the existing information about methyltransferases is obtained by reverse genetics and researchers may use forward genetics to confirm their results on how methyltransferases would function in plant development.

ACKNOWLEDGMENTS

This work was supported by the research grants from the Ministry of Sciences and Technology (973 project number: 2007CB108700) and the National Science Foundation of China (30330310).

REFERENCES

Arnaud P, Feil R (2006). MEDEA takes control of its own imprinting. Cell, 124: 468-470.

Aufsatz W, Mette MF, Matzke AJ, Matzke M (2004). The role of MET1 in RNA-directed *de-novo* and maintenance methylation of CG dinucleotides. Plant Mol. Biol. 54: 793-804.

Bartee L, Bender J (2001). Two *Arabidopsis* methylation-deficiency mutations confer only partial effects on a methylated endogenous gene family. Nucleic Acids Res. 29: 2127-2134.

Bartee L, Malagnac F, Bender J (2001). *Arabidopsis* cmt3 chromomethylase mutations block non-CG methylation and silencing of an endogenous gene. Genes Dev. 15: 1753-1758.

Berger F, Chaudhury A (2009). Parental memories shape seeds. Trends Plant Sci. 14: 550-556.

Bestor TH (2000). The DNA methyltransferases of mammals. Hum. Mol.

Genet. 9: 2395-240.
Cao X, Aufsatz W, Zilberman D, Mette MF, Huang MS, Matzke M, Jacobsen SE (2003). Role of the DRM and CMT methyltransferases in RNA-directed DNA methylation. Curr. Biol. 13: 2212-2217.
Cao X, Jacobsen SE (2002a). Locus-specific control of asymmetric and CpNpG methylation by the DRM and CMT3 methyltransferase genes. Proc. Natl. Acad. Sci. 99: 16491-16498.
Cao X, Jacobsen SE (2002b). Role of the *Arabidopsis* DRM methyltransferases in *de novo* DNA methylation and gene silencing. Curr. Biol. 12: 1138-1144.
Cao X, Springer NM, Muszynski MG, Phillips RL, Kaeppler S, Jacobsen SE (2000). Conserved plant genes with similarity to mammalian *de novo* DNA methyltransferases. Proc. Natl. Acad. Sci 97: 4979-4984.
Chan SWL, Henderson IR, Jacobsen SE (2005). Gardening the genome:DNA methylation in *Arabidopsis thaliana*. Genetics, 6: 351-360.
Chan SWL, Henderson IR, Zhang X, Shah G, Chien JSC, Jacobsen SE (2006). RNAi, DRD1, and histone methylation actively target developmentally important non-CG DNA methylation in *Arabidopsis*. PloS. Genet. 2: 791-797.
Chan SWL, Zilberman D, Xie Z, Johansen LK, Carrington JC, Jacobsen SE (2004). RNA silencing genes control *de novo* DNA methylation. Science, 303: p. 1336.
Chen M, Ha M, Lackey E, Wang J, Chen ZJ (2008). RNAi of *met1* reduces DNA methylation and induces genome-specific changes in gene expression and centromeric small RNA accumulation in *Arabidopsis* allopolyploids. Genetics, 178: 1845-1858.
Cokus SJ, Feng S, Zhang X, Chen Z, Merriman B, Haudenschild CD, Pradhan S, Nelson SF, Pellegrini M, Jacobsen SE (2008). Shotgun bisulfite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. Nature, 452: 215-219.
Eden A, Gaudet F, Waghmare A, Jaenisch R (2003). Chromosomal instability and tumors promoted by DNA hypomethylation. Science, 300: p. 455.
Eissenberg JC (2001). Molecular biology of the chromodomain: an ancient Chromatin module comes of age. Gene. 275: 19-29.
Finnegan EJ, Dennis ES (1993). Isolation and identification by sequence homology of a putative cytosine methyltransferase from *Arabidopsis thaliana*. Nucleic Acids Res. 21: 2383-2388.
Finnegan EJ, Kovac KA (2000). Plant DNA methyltransferases. Plant Mol. Biol. 43: 189-201.
Finnegan EJ, Peacock WJ, Dennis ES (1996). Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. Proc. Natl. Acad. Sci. 93: 8449-8454.
FitzGerald J, Luo M, Chaudhury A, Berger F (2008). DNA methylation causes predominant maternal controls of plant embryo growth. PLos. One, 3: p. 2298.
Fransz P, ten Hoopen R, Tessadori F (2006). Composition and formation of heterochromatin in *Arabidopsis thaliana*. Chromosome Res. 14: 71-82.
Fuchs J, Demidov D, Houben A, Schubert I (2006). Chromosomal histone modification patterns-from conservation to diversity. Trends Plant Sci. 11: 199-208.
Gehring M, Henikoff S (2007). DNA methylation dynamics in plant genomes. Biochim. Biophys. Acta. 1769: 276-286.
Genger RK, Kovac KA, Dennis ES, Peacock WJ, Finnegan EJ (1999). Multiple DNA methyltransferase genes in *Arabidopsis thaliana*. Plant Mol. Biol. 41: 269-278.
Goll MG, Bestor TH (2005). Eukaryotic cytosine methyltransferases. Annu. Rev. Biochem. 74: 481-514.
Henderson IR, Jacobsen SE (2008). Tandem repeats upstream of the *Arabidopsis* endogene *SDC* recruit non-CG DNA methylation and initiate siRNA spreading. Genes Dev. 22: 1597-1606.
Henikoff S, Comai L (1998). A DNA methyltransferase homolog with a chromodomain exists in multiple polymorphic forms in *Arabidopsis*. Genetics, 149: 307-318.
Jackson JP, Lindroth AM, Cao X, Jacobsen SE (2002). Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature, 416: 556-560.
Jacobsen SE, Sakai H, Finnegan EJ, Cao X, Meyerowitz EM (2000). Ectopic hypermethylation of flower-specific genes in *Arabidopsis*. Curr. Biol. 10: 179-186.
Jullien PE, Kinoshita T, Ohad N, Berger F (2006). Maintenance of DNA methylation during the *Arabidopsis* life cycle is essential for parental imprinting. Plant Cell, 18: 1360-1372.
Kakutani T (2002). Epialleles in plants: Inheritance of epigenetic information over generations. Plant Cell. Physiol. 43: 1106-1111.
Kankel MW, Ramsey DE, Stokes TL, Flowers SK, Haag JR, Jeddeloh JA, Riddle NC, Verbsky ML, Richards EJ (2003). *Arabidopsis* MET1 cytosine methyltransferase mutants. Genetics, 163: 1109-1122.
Kidwell MG, Lisch DR (2001). Perspective: transposable elements, parasitic DNA, and genome evolution. Evolution, 55: 1-24.
Kinoshita T, Miura A, Choi Y, Kinoshita Y, Cao X, Jacobsen SE, Fischer RL, Kakutani T (2004). One-way control of FWA imprinting in *Arabidopsis* endosperm by DNA methylation. Science, 303: 521-523.
Li E (2002). Chromatin modification and epigenetic reprogramming in mammalian development. Nat. Rev. Genet. 3: 662-673.
Lindroth AM, Cao X, Jackson JP, Zilberman D, McCallum CM, Henikoff S, Jacobsen SE (2001). Requirement of chromomethylase 3 formaintenance of CpXpG methylation. Science, 292: 2077-2080.
Lindroth AM, Shultis D, Jasencakova Z, Fuchs J, Johnson L, Schubert D, Patnaik D, Pradhan S, Goodrich J, Schubert I, Jenuwein T, Khorasanizadeh S, Jacobsen SE (2004). Dual histone H3 methylation marks at lysines 9 and 27 required for interaction with chromomethylase 3. EMBO J. 23: 4286-4296.
Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, Ecker JR (2008). Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. Cell, 133: 523-536.
Malagnac F, Bartee L, Bender J (2002). An *Arabidopsis* SET domain protein required for maintenance but not establishment of DNA methylation. EMBO J. 21: 6842-6852.
Mathieu O, Probst AV, Paszkowski J (2005). Distinct regulation of Histone H3 methylation at lysines 27 and 9 by CpG methylation in *Arabidopsis*. EMBO J. 24: 2783-2791.
Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszkowski J (2007). Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG Methylation. Cell, 130: 851-862.
Miki D, Shimamoto K (2008). *De novo* DNA methylation induced by siRNA targeted to endogenous transcribed sequences is gene-specific and *OsMet1*-independent in rice. Plant J. 56: 539-549.
Mueller TD, Feigon J (2002). Solution structures of UBA domains reveal a conserved hydrophobic surface for protein-protein interactions. J. Mol. Biol. 319: 1243-1255.
Papa CM, Springer NM, Muszynski MG, Meeley R, Kaeppler SM (2001). Maize chromomethylase Zea methyltransferase 2 is required for CpNpG methylation. Plant Cell, 13: 1919-1928.
Pavlopoulou A, Kossida S (2007). Plant cytosine-5 DNA methyltransferases: structure, function, and molecular evolution. Genomics, 90: 530-541.
Penterman J, Uzawa R, Fischer RL (2007). Genetic interactions between DNA demethylation and methylation in *Arabidopsis.* Plant Physiol. 145: 1549-1557.
Pérez-Hormaeche J, Potet F, Beauclair L, Le Masson I, Courtial B, Bouché N, Lucas H (2008). Invasion of the *Arabidopsis* genome by the tobacco retrotransposon Tnt1 is controlled by reversible transcriptional gene silencing. Plant Physiol. 147: 1264-1278.
Pien S, Grossniklaus U (2007). *Polycomb* group and *trithorax* group proteins in *Arabidopsis*. Biochim. Biophys. Acta. 1769: 375-382.
Rangwala SH, Richards EJ (2007). Differential epigenetic regulation within an *Arabidopsis* retroposon family. Genetics, 176: 151-160.
Richards EJ (1997). DNA methylation and plant development. Trends Genet. 13: 319-323.
Saitou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.
Saze H, Mittelaten Scheid O, Paszkowski J (2003). Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. Nat. Genet. 34: 65-69.
Saze H, Shiraishi A, Miura A, Kakutani T (2008). Control of genic DNA methylation by a jmjC domain-containing protein in *Arabidopsis thaliana*. Science, 319: 462-465.
Singh A, Zubko E, Meyer P (2008). Cooperative activity of DNA methyltransferases for maintenance of symmetrical and non-symmetrical cytosine methylation in *Arabidopsis thaliana*. Plant J. 56: 814-823.

Soppe WJ, Jacobsen SE, Alonso-Blanco C, Jackson JP, Kakutani T, Koornneef M, Peeters AJ (2000). The late flowering phenotype of fwa mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. Mol. Cell. 6: 791-802.

Soppe WJ, Jasencakova Z, Houben A, Kakutani T, Meister A, Huang MS, Jacobsen SE, Schubert I, Fransz PF (2002). DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in *Arabidopsis*. EMBO J. 21: 6549-6559.

Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Tariq M, Saze H, Probst AV, Lichota J, Habu Y, Paszkowski J (2003). Erasure of CpG methylation in *Arabidopsis* alters patterns of Histone H3 methylation in heterochromatin. Proc. Natl. Acad. Sci. 100: 8823-8827.

Vanyushin BF (2005). Enzymatic DNA methylation is an epigenetic control for genetic functions of the cell. Biochemistry, 70: 488-499.

Vaughn MW, Tanurd Ić M, Lippman Z, Jiang H, Carrasquillo R, Rabinowicz PD, Dedhia N, McCombie WR, Agier N, Bulski A, Colot V, Doerge RW, Martienssen RA (2007). Epigenetic natural variation in *Arabidopsis thaliana*. PloS Biol. 5: 1617-1629.

Villar-Garea A, Fraga MF, Espada J, Esteller M (2003). Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. Cancer Res. 63: 4984-4989.

Weber M, Schübeler D (2007). Genomic patterns of DNA methylation: targets and function of an epigenetic mark. Curr. Opin. Cell Biol.19: 273-280.

Xiao W, Brown RC, Lemmon BE, Harada JJ, Goldberg RB, Fischer RL (2006). Regulation of seed size by hypomethylation of materna1 and paternal genomes. Plant Physiol. 142: 1160-1168.

Xiao W, Gehring M, Choi Y, Margossian L, Pu H, Harada JJ, Goldberg RB, Pennell RI, Fischer RL (2003). Imprinting of the MEA Polycomb gene is controlled by antagonism between MET1 methyltransferase and DME glycosylase. Dev. Cell. 5: 891-901.

Zhang X (2008). The epigenetic landscape of plants. Science, 320: 489-492.

Zhang X, Jacobsen SE (2006). Genetic analyses of DNA methyltransferases in *Arabidopsis thaliana*. Cold Spring. Harb. Symp. Quant. Biol. 71: 439-447.

Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, Chen H, Henderson IR, Shinn P, Pellegrini M, Jacobsen SE, Ecker JR (2006). Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. Cell, 126: 1189-1201.

Zhu J, Kapoor A, Sridhar VV, Agius F, Zhu JK (2007). The DNA glycosylase/lyase ROS1 functions in pruning DNA methylation patterns in *Arabidopsis*. Curr. Biol. 17: 54-59.

Zilberman D, Cao X, Johansen LK, Xie Z, Carrington JC, Jacobsen SE (2004). Role of *Arabidopsis* Argonaute 4 in RNA-directed DNA methylation triggered by inverted repeats. Curr. Biol. 14: 1214-1220.

Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S (2007). Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. Nat. Genet. 39: 61-69.