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Review

Physiological functions of plant DNA methyltransferases*

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Abstract Epigenetic regulation is defined as mechanisms that control gene expression without altering base sequences. Cytosine methylation, chromatin remodeling, and modifications at the N-termini of core histones are key factors in this regard. Epigenetic modifications are found throughout the eukaryotes, suggesting that they developed at an early stage in biological evolution, although actual molecular mechanisms show considerable variation among species. In particular, plants are unique in establishment and maintenance of epigenetic states, as exemplified by species-specific enzymes that catalyze DNA methylation. Since the function and diversity of DNA methyltransferases in individual species are not fully understood, I here summarize recent findings in plant epigenetics, focusing on DNA methyltransferases classified into three major groups. Their possible biological functions are also discussed with reference to histone modification and chromatin remodeling.

Key words: DNA methylation, epigenetics, histone modification.

Among factors that regulate gene expression in eukaryotes, DNA methylation, chromatin remodeling and modification of N-termini of core histones are considered to play key roles. All of these are epigenetic and recent studies have provided much information on the molecular basis of such modification and interrelationships between DNA and chromatin modification in regulation of normal development. Epigenetic modification in plants is particularly responsive to environmental stimuli. In this article, I briefly summarize current knowledge of DNA methylation in plants, and discuss its biological significance.

Methylation of DNA

The most commonly modified base in DNA among the eukaryotes through animals and plants is 5-methylcytosine (m^5C) (Yoder and Walsh et al. 1997) (Figure 1), first found in 1951 and confirmed to be a minor base in DNA (Wyatt 1951). Its proportion of the total bases varies among organisms, ranging between less than 0.25% in bacteria up to 7% in plants (Hall 1971). Initial ideas on its physiological function were focused on protection of host DNA from degradation by the so-called restriction-modification system (Kuhnlein and Arber 1972), consisting of a DNA methyltransferase and a corresponding restriction endonuclease, identified in many bacteria (Smith and Kelly 1984).

In eukaryotes, m^5C occurs in retroelements (Yoder and Walsh et al. 1997), and frequently is located in CpG islands within promoter regions of genes (reviewed in Bird 1986). Changes affect suppression of invading sequences of DNA and management of endogenous gene expression via condensation of chromatin structure. In mammals, m^5C is also associated with development of cancer (reviewed in Robertson and Wolffe 2000; Jones and Baylin 2002; Ehrlich 2000, 2003), X-chromosome inactivation (Panning and Jaenisch 1996), genomic imprinting (reviewed in Ferguson-Smith and Surani 2001), tissue specific gene expression (Futscher et al. 2002), and heterochromatin formation (Urnov and

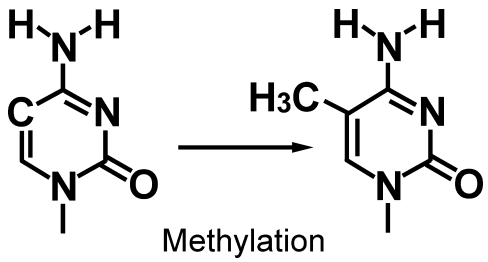

Figure 1. Structure of methylated cytosine. Unmethylated cytosine (left) is methylated at the C-5 position of the aromatic ring by a cytosine methyltransferase, yielding m^5C (right).

Abbreviations: AdoMet, S-adenosyl-L-methionine; CMT, chromomethylase; Dnmt, DNA methyltransferase; DRM, domains rearranged methyltransferase; MBDs, methyl-CpG-binding domain proteins; MET1, Methyltransferase 1; m^5C, 5-methylcytosine; siRNA, small interference RNA molecules; UBA, ubiquitin-association.

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Wolffe 2001).

Methylation of cytosine residues in DNA is enzymatically catalyzed by DNA methyltransferases, which transfer a methyl-group from S-adenosyl-L-methionine (AdoMet) to the 5-position. The methylation mechanism of DNA was first determined by Holliday and Pugh (1975) and Riggs (1975) and patterns of methylated bases were proposed to be heritable, assuming that once established by a *de novo* DNA methyltransferase activity, methylation could be faithfully maintained by maintenance DNA methyltransferase activity recognizing newly replicated, hemimethylated DNA, a DNA duplex methylated in one strand but unmethylated in the other (Figure 2). With this hypothesis, two different activities of methyltransferase were predicted and subsequent progress in analysis of DNA methylation has provided much of evidence for the model. CpG doublets, which are methylated to a high level in mammalian cells, are frequently seen in retroelements and within promoter regions of particular genes, which are called CpG islands. The CpG doublet is symmetrical, being often methylated in both strands.

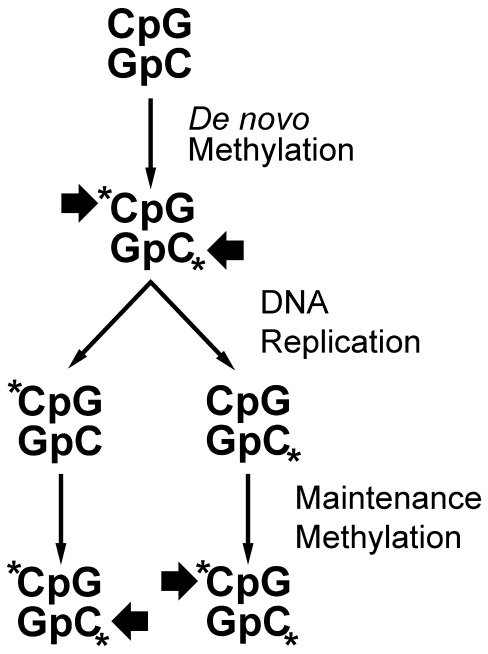

Figure 2. Symmetric cytosine methylation. Cytosines in unmethylated CpG doublets (top) are targeted by a *de novo* methyltransferase, which methylates unmethylated cytosines (middle). After semiconservative DNA replication, newly synthesized strand is base paired with a parental methylated strand (middle). Symmetry is restored by maintenance methyltransferase (bottom), which targets half-methylated CpG sites, but does not methylate unmethylated cytosines.

DNA methylation in mammals

Mammalian DNA methyltransferase 1 (Dnmt1), the first eukaryotic cytosine methyltransferase to be characterized, is related to bacterial type-II cytosine restriction methyltransferases (Bestor et al. 1988). It displays a strong preference for hemimethylated DNA, with a 30-fold higher activity than for unmethylated DNA *in vitro* (Yoder and Soman et al. 1997), and completes half-methylated sites after semiconservative DNA replication, restoring symmetry of methylated cytosines. It is therefore referred to as a maintenance methyltransferase.

Ten years after its discovery, examples of another type of DNA methyltransferase, targeting unmethylated DNA, were discovered. These were named *de novo* methyltransferases, 3a (Dnmt3a) and 3b (Dnmt3b) (Okano et al. 1998) and failure to establish methylation patterns during embryogenesis was found with mutants of the two enzymes, along with impaired development (Okano et al. 1999). Mammalian cells exhibit cell type specific DNA methylation patterns, generated by *de novo* and maintenance DNA methyltransferase activities during every DNA replication. However, patterns of methylation are completely erased during gametogenesis, allowing creation of new patterns during early developmental stage in progeny (Reviewed in Bird 2002).

Epigenetic inheritance is defined as a heritable change in gene function that cannot be explained by changes in DNA sequence (Russo et al. 1996). The mechanism of epigenetic regulation of gene expression has been intensively investigated over the last decade, and cytosine methylation is now generally accepted to play a critical role. The m^5C is considered as a hallmark for transcriptional inactivation involving methyl-CpG-binding domain proteins (MBDs) (Wakefield et al. 1999), the latter recruiting various protein complexes that consist of histone deacetylase and ATP-dependent chromatin remodeling factors (Zhang et al. 1999). MBDs are also associated with histone methyltransferase activity (Fuks et al. 2003). Both deacetylation and methylation of histone N-termini lead to condensation of chromatin and transcriptional repression (Jenuwein and Allis 2001). It has been reported that histone methylation is essential for DNA methylation in *Neurospora crassa* (Tamaru and Selker 2001), and a similar relationship has been established in mammals (Lehnertz et al. 2003) and in *Arabidopsis* (Jackson et al. 2002). The available observations thus strongly suggest a functional relationship between DNA methylation and chromatin modification. Indeed, studies have provided substantial evidence that DNA methyltransferases directly interact with histone modification enzymes (Fuks et al. 2000; Robertson et al. 2000; Rountree et al. 2000) and with MBDs (Kimura and Shiota 2003).

DNA methylation in plants

The level of plant DNA methylation is generally higher

Table 1. Target sequence of DNA methylation in model organisms.

Species	Methylatedsequence		
	mCpG	mCpNpG	mCpNpN
S. cerevisiae	–	–	–
S. pombe	–	–	–
N. crassa	+	+	+
D. melanogaster	–	–	–
A. thaliana	++	++	++
M. musculus	++	+	–

Relative frequencies of methylated cytosines in each context are shown. N stands for any base.

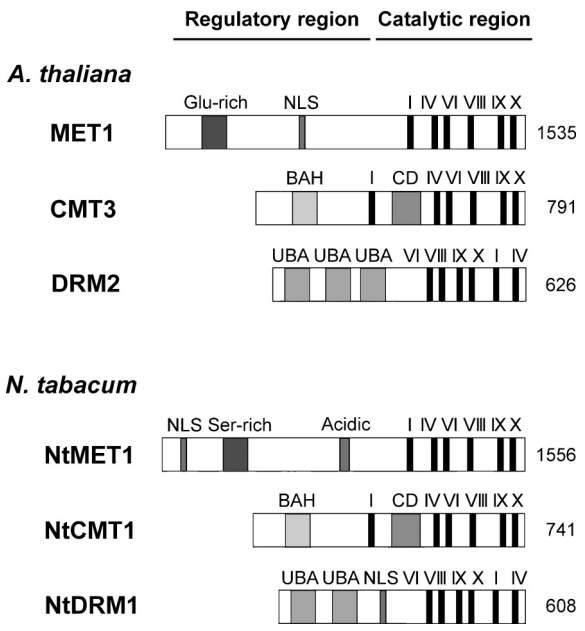

Figure 3. Schematic diagrams of plant DNA methyltransferases. The size of each protein is indicated in amino acid numbers, and conserved motifs in the catalytic region are indicated by closed boxes with numbers. Specific regions in the regulatory region are indicated by shaded boxes with appropriate names. Glu-rich, glutamine rich acidic region; BAH, bromo-adjacent homology domain; CD, chromodomain; NLS, nuclear localization signal; UBA, ubiquitin association domain. Sequence data are obtained from the data base; accession numbers are P34881 (*MET1*), AF383170 (*CMT3*), AF240695 (*DRM2*), AB030726 (*NtMET1*), AB032538 (*NtCMT1*) and AB087883 (*NtDRM1*).

than in mammals. The locations of m^5C also differ, being found not only in CpG dinucleotides, but also in CpNpG trinucleotides and other sequence contexts (Table 1). Such specific methylation patterns in plants are created by specific DNA methyltransferases that are unique to plants (Figure 3). Methylation of cytosines in CpG and CpNpG, often referred as symmetric cytosines, is catalyzed by maintenance methyltransferases, and can be transmitted through meiosis to progeny. However, cytosines in CpNpN, referred as non-symmetrical, are not methylated by maintenance type enzymes, and currently it is not clear whether or not non-symmetrical methylation patterns can be maintained. Genetic and biochemical studies have been intensively performed to clarify mechanisms and physiological significance of DNA methylation and responsible methyltransferases in plants and in the *Arabidopsis* genome there are at least ten genes encoding DNA methyltransferases that can be divided into three families (Finnegan and Kovac 2000) (Table 2). The first is exemplified by methyltransferase 1 (MET1). The second family contains chromomethylase (CMT) and the third features the domains rearranged methyltransferase (DRM). These enzymes have already been identified from a variety of plants, including maize, tobacco and rice (Finnegan and Denis 1993; Genger et al. 1999; Olhoft 1998; Steward et al. 2000; Nakano et al. 2000; Bernacchia et al. 1998; Pradhan et al. 1998; Henikoff and Comai 1998; Rose et al. 1998; Lindroth et al. 2001; Bartee et al. 2001; Tompa et al. 2002; Papa et al. 2001; Cao et al. 2000; Wada et al. 2003) (Table 2). In the following sections, I briefly summarize their properties and also findings with mutants for chromatin remodeling factors, which govern the global methylation status created by these enzymes.

Methyltransferase 1

Methyltransferase 1 (MET1) is considered to be an ortholog of mammalian Dnmt1 (Finnegan and Dennis 1993), having a large N-terminal regulatory domain and a C-terminal catalytic domain (Finnegan and Dennis 1993). There are four genes in the *MET1* family in *Arabidopsis*, and genetic analysis has suggested that their products function in maintenance of global genomic methylation (Finnegan et al. 1996; Ronemus et al. 1996). *MET1* mutants exhibit drastically decreased DNA methylation levels and morphological abnormalities (Finnegan et al. 1996; Ronemus et al. 1996; Kankel et al. 2003). A similar reduction of global methylation and altered phenotypes were also observed in transgenic tobacco plants in which DNA methylation levels were suppressed by expression of anti-sense *NtMET1*, which encodes a maintenance DNA methyltransferase in tobacco plants (Nakano et al. 2000). Subsequent screening of genes whose expression was specifically affected in these transgenic plants, revealed more than half to be related to stress responses. The finding indicated that maintenance of DNA methylation is critical for concerted regulation of gene expression (Wada et al. 2004).

MET1 is also known to be necessary for the maintenance of methylation during gametogenesis. *MET1* gene loss in megaspores leads to passive DNA demethylation during megagametogenesis (Saze et al. 2003), which resembles the mammalian *dnmt1* knockout case featuring passive demethylation during early development (Okano et al. 1999). Maintenance methylation by MET1 is reported to be necessary for maintenance of parent-of-origin specific expression of, for example, *MEDEA* (*MEA*) and *FWA* in endosperm

Table 2. Target sequence and predicted function of plant DNA methyltransferases.

Classification	Gene Name	Species	Target sequence	Function	References
MET1	*MET1*, *DDM2*	*A. thaliana*	CpG	Maintenance of mCpG	Finnegan and Denis 1993
	MET2	*A. thaliana*			Genger et al. 1999
	METIIb	*A. thaliana*			Genger et al. 1999
	MET3	*A. thaliana*			Genger et al. 1999
	ZmMET1	*Z. mays*	CpG		Olhoft 1998, Steward et al. 2000
	NtMET1	*N. tabacum*	CpG, CpCpG		Nakano et al. 2000
	CMET1-5	*D. carota*	CpNpG		Bernacchia et al. 1998
	CMET2-21	*D. carota*			Bernacchia et al. 1998
	PMET	*P. sativum*	CpG, CpWpG		Pradhan et al. 1998
MET2	*DMT11*	*A. thaliana*			
	ZMET4	*Z. mays*			
CMT	*CMT1*	*A. thaliana*	Nonfunctional		Henikoff and Comai 1998
	CMT2	*A. thaliana*			Rose et al. 1998
	CMT3	*A. thaliana*	CpNpG at *SUP, PAI* locus and *Athila* type retrotransposon		Lindroth et al. 2001, Bartee et al. 2001, Tompa et al. 2002
	ZMET2	*Z. mays*	CpNpG at knob region		Papa et al. 2001
	NtCMT1	*N. tabacum*	Unknown		
DRM	*DRM1*	*A. thaliana*	Non-CpG	Induction of silencing	Cao et al. 2000
	DRM2	*A. thaliana*	Non-CpG	Induction of silencing	Cao et al. 2000
	DRM3	*A. thaliana*			
	ZMET3	*Z. mays*			Cao et al. 2000
	DMT106	*Z. mays*			
	NtDRM1	*N. tabacum*	CpHpG, CpHpH		Wada et al. 2003
DNA glycosilase	*DME*	*A. thaliana*	Imprinted genes, *FWA* and *MEA*		Choi et al. 2002
	ROS	*A. thaliana*	mCpCpG of silent transgene and homologous endogene		Gong et al. 2002
SWI2/SNF2	*DDM1*	*A. thaliana*	CpG, CpNpG		Miura et al. 2001
			CACTA type retrotransposon		Kato et al. 2003
	DRD	*A. thaliana*	CpNpN		Kanno et al. 2004

N stands for any base; H stands for A, T or C; W stands for A or T.

(Xiao et al. 2003; Kinoshita et al. 2004). MET1 is needed for methylation maintenance and silencing of transgenes (Jones et al. 2001). The results suggest that maintenance of DNA methylation is indispensable for regulation of gene expression and normal plant development.

Chromomethylase

Chromomethylase (CMT) is unique to the plant kingdom and controls non-CpG methylation (Cao and Jacobsen 2002a), *CMT*-knockouts exhibiting pleiotropic developmental abnormalities (Cao and Jacobsen 2002a). There are three *CMT* related genes in *Arabidopsis*: *CMT1*, *CMT2* and *CMT3* (Table 2). All contain a characteristic chromodomain, which is often seen in chromatin-related proteins (Cavalli and Paro 1998). *CMT1* may be nonfunctional in several ecotypes of *Arabidopsis* through insertion of an intact retrotransposon (Henikoff and Comai 1998). *CMT3* was isolated by screening for mutations that depress the silencing of the heavily methylated *Arabidopsis SUPERMAN* (*SUP*) locus, and has been described to be responsible for maintenance of cytosine methylation at CpNpG (where N is A, T, C or G) sites of *SUP* (Lindroth et al. 2001). *CMT3* was also reported to be needed for maintenance of repeat *phosphoribosyl-anthranilate-isomerase* (*PAI*) loci methylation in a Wassilewskija ecotype (Bartee et al. 2001) and to be responsible for maintenance of retrotransposon methylation (Tompa et al. 2002). In both cases, the methylation targets are CpNpG sites. The maize *CMT* gene, *ZMET2*, has been shown to be involved in CpNpG methylation of the knob regions of constitutive heterochromatin of chromosomes (Papa et al. 2001). It can thus be concluded that CMT3 maintains CpNpG methylation in heterochromatin and silencing of methylated loci. However, the mechanism by which CMT3 recognizes targets is not clear. Recent analyses of chromodomain in catalytic motifs suggested that it may recognize transcriptionally silent chromosomes. CMT3 possibly directly interacts with heterochromatin by binding to the N-terminus of histone H3, whose lysines at positions 9 (K9) and 27 (K27) are methylated. Since such methylation is a characteristic modification of transcriptionally silent heterochromatinic regions (Lindroth et al. 2004), it is probable that CMT3

recognizes heterochromatinic hallmarks, resulting in methylation of their cytosines.

Domains rearranged methyltransferase

Amino acid sequence analyses have indicated that the domains rearranged methyltransferase (DRM) type has catalytic motifs resembling mammalian *de novo* enzymes such as Dnmt3 (Cao et al. 2000), although they differ in possessing a characteristic rearrangement in catalytic motifs, between I–V and VI–X. The other characteristic feature of proteins belonging to the DRM family is the presence of two or three ubiquitin-association (UBA) domains, which function in protein-protein interactions (Hofmann and Bucher 1996). *Arabidopsis* has at least three *DRM* genes: *DRM1*, *DRM2* and *DRM3* and genetic analysis with T-DNA insertion lines suggested that *DRM1* and *DRM2* might be responsible for methylation of cytosines in inverted-repeat transgenes at both CpNpG and CpNpN sites (Cao and Jacobsen 2002b). We previously reported isolation and enzymatic characterization of a tobacco DRM (Wada et al. 2003). The enzyme expressed in insect cells could be shown to preferentially methylate cytosine residues in CpNpN and also CpNpG (where N is A, T, or C), providing concrete evidence for the predicted functions.

Methylation of symmetric CpG in mammals and plants is mainly mediated by DNA methyltransferases of maintenance type, which, by recognizing m^5CpG in the mother strand, methylate opposite CpGs in newly replicated daughter strands after semiconservative DNA replication. This essentially results in the maintenance of the same methylation pattern throughout cell division. In contrast, the methylation pattern at asymmetric CpNpN can not usually be maintained after DNA replication (Figure 4). In this context, asymmetric cytosine methylation can not be termed an epigenetic modification in itself, because of the lack of maintenance ability during cell division. Nevertheless, asymmetric cytosine methylation has been known to play a critical role in gene expression, for example, occurring in *de novo* fashion during RNA silencing, known as co-suppression, and in gene silencing or with RNAi (Meyer and Heidmann 1994; Wassenegger and Pelissier 1998; Wassenegger 2000). It has also been reported to be associated with epigenetically silenced endogenes (Jacobsen and Meyerowitz 1997), and further analyses confirmed this, indicating that DRM functions in RNA-directed DNA methylation (RdDM). A *drm* mutant was found to suppress *de novo* methylation directed by generation of small interference RNA molecules (siRNA) (Cao et al. 2003; Chan et al. 2004; Zilberman et al. 2004). The mechanism by which DRM recognizes siRNA and then methylates corresponding genomic loci during RNA silencing remains to be determined.

Figure 4. Dwarf phenotype of transgenic tobacco plants transformed with antisense *NtMET1*. At maturity, lines #86 (right) and #62 (middle) carrying the antisense *NtMET1* apparently showed dwarfism in comparison with the non-transformed control plant (left).

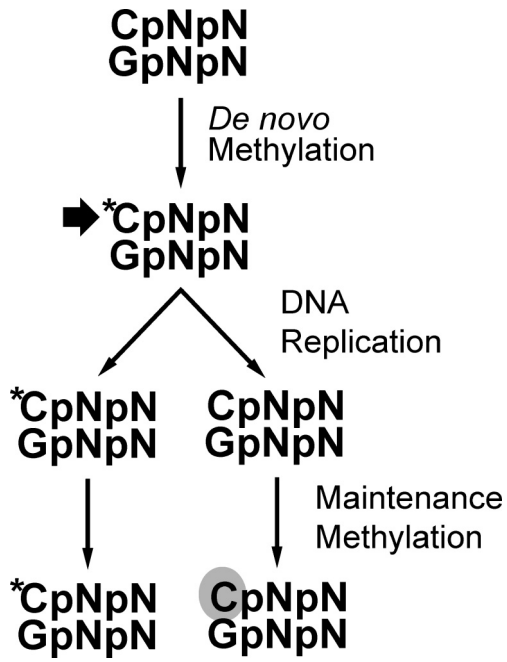

Figure 5. Asymmetric cytosines methylation. Cytosines located at asymmetric sequence (top) are often methylated by an asymmetric *de novo* cytosine methyltransferase (upper). After semiconservative DNA replication, newly synthesized strand is base paired with a parental methylated strand (middle). However, because of its sequence asymmetry, methylation is not restored by maintenance methyltransferase (bottom).

DDM1

In addition to the above-mentioned DNA methyltransferases, a gene encoding DECREASED IN DNA METHYLATION1 (DDM1), which belongs to the SWI2/SNF2 family of chromatin remodeling factors, has been shown to be necessary for maintenance of DNA methylation (Jedelloh et al. 1999; Kakutani et al. 1999; Dennis et al. 2001). A *ddm1* mutant was originally isolated as a mutation that reduced m^5C levels by 70% (Vongs et al. 1993) and subsequent extensive analyses have revealed that *DDM1* is essential for inactivation of transposable elements (Miura et al. 2001), as well as for maintenance of heterochromatic regions (Mathieu et al. 2003; Lippman et al. 2004). Recombinant DDM1 can induce the movement of histone octamers along DNA in an ATP-dependent manner (Brzeski and Jerzmanowski 2003). A putative chromatin remodeling factor, *DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1* (*DRD1*) may also be necessary for non-CpG methylation (Kanno et al. 2004). *DRD1* encodes a plant-specific ATP-dependent chromatin remodeling factor, a SWI2/SNF2-like protein, and a *drd1* mutant was recently shown to lack non-CpG methylation induced by RNA-mediated silencing (Kanno et al. 2004). The available data clearly indicate that mutations in chromatin remodeling factors affect DNA methylation, probably through chromatin structures. The underlying molecular mechanisms by which chromatin remodeling interacts with DNA methylation and/or DNA methyltransferase actions has yet to be clarified.

Reprogramming of DNA methylation patterns

In mammals, the epigenetic status is systematically reconstructed in every individual during development, and patterns of DNA methylation are cell-type specific. This specificity was shown to be closely related to selective repression of gene expression, resulting in determination of cell properties. DNA methylation is also associated with expression of imprinted genes, which are expressed predominantly from one allele in a parent-of-origin-specific manner. The suppressed allele is often methylated and the mechanism by which DNA methylation patterns are established is proposed to involve erasure of existing patterns in germ cells, followed by new *de novo* methylation during imprinting. Demethylation and subsequent remethylation result in reprogramming of cell-type-specific methylation patterns, and consequently, in genomic imprinting (Reik et al. 2001; Surani 2001).

In higher plants, there has been no clear evidence of resetting of the epigenetic status, although several cases of genomic imprinting have been reported. However, recent analyses showed that plant imprinting apparently does not occur through resetting of methylation. Instead, it may be established by removal of m^5C rather than by *de novo* methylation and locus specific excision of m^5C may be catalyzed by DNA glycosylases. For example, there is evidence that the DEMETER (DME) DNA glycosylase is responsible for expression of two imprinted loci in endosperm in a maternal allele specific manner. DME removes m^5C (Choi et al. 2002) and reactivates the *FWA* for the homeodomain protein (Kinoshita et al. 2004). It also releases the silent status of *MEA* for polycomb by nicking its promoter (Xiao et al. 2003). Partial demethylation for establishment of imprinting supports the idea that plants are not equipped with a large-scale system for resetting the epigenetic status. There is also no clear evidence that plants possess a mechanism to reestablish DNA methylation. In the *ddm1* mutant, the demethylated state is not easily restored even on introduction of a wild-type *DDM1* gene. In rice initially treated with a demethylating reagent, azaC, dwarfism was induced with a concomitant reduction of m^5C content, which was not restored after several generations. It can be concluded that the DNA methylation pattern is not erased during gametogenesis, and therefore that it is not reestablished in every generation.

In contrast to mammalian DNA methylation, which is strictly controlled during cell division and transmission to progeny under ordinary conditions, plant DNA methylation is rather flexible. For example, m^5C in plant DNA can be excised by DNA glycosylase, DME, as described above. Furthermore, *Repressor of silencing 1* (*ROS1*) encodes a DNA glycosylase/lyase which releases methylation and silencing of transgene and endogenous homologous loci (Gong et al. 2002). We have established that the DNA methylation status changes in response to environmental stimuli. For example, cold treatment of maize seedlings resulted in a global demethylation of root genomic DNA, particularly in nucleosome core regions (Steward et al. 2002). Pathogen attack to tobacco plants was found to simultaneously induce demethylation of a particular gene and its transcripts (Wada et al. 2004). These observations support the idea that the methylation status of plant DNA is not stable and that it may routinely change under certain circumstances, such as environmental stress.

Reprogramming of chromatin structure

Change of chromatin structure has been reported to occur in the flowering factor gene locus of *Arabidopsis* upon cold exposure (Finnegan et al. 2004). It is well known that prolonged exposure to cold temperature is required for the appropriate timing of flowering in several plant species, this process being called

vernalization. Cold treatment, for example, prevents accumulation of transcripts for *FLOWERING LOCUS C* (*FLC*), a MADS-box transcriptional factor which acts as a flowering repressor (Michael and Amasino 1999). *FLC* repression is maintained epigenetically through cell division, and its molecular mechanism has now been found to involve histone deacetylation in the region of the large first intron (He et al. 2003; Ausin et al. 2004). Site specific histone methylation could also be shown to be associated with reduced gene expression in many species (Rea et al. 2000), and this is the case with *FLC*. Methylation of K27 located in the N-terminus of histone H3 appears to be involved in *FLC* silencing (Bastow et al. 2004), this being dependent on VRN2, a polycomb group (PcG) protein (Sung and Amasino 2004) associated with histone modification enzymes in *Drosophila* and mammalian cells (Cao et al. 2002; Müller et al. 2002; Kuzmichev et al. 2002). Whatever the case, the molecular mechanism of vernalization is partly mediated through down regulation of *FLC* gene expression via an alteration of histone modification at its locus. Thus environmental stimuli can change epigenetic information, although it is not clear at present to what extent histone modification might be reset or transmitted to the next generation. Although DNA methylation is considered to be involved in imprinting and resetting of certain genes, whether changes in histone modification at the *FLC* locus during vernalization correlate with DNA methylation is currently not clear.

Concluding remarks

An interplay between DNA methylation and chromatin modification appears to be essential for epigenetic regulation, which is stably maintained through mitosis, and under certain circumstances, through meiosis to descendants. During evolution, it is to be expected that some of the underlying mechanisms might have become diversified and others conserved. For example, while the epigenetic phenomenon itself is common among animals and plants, the mechanism for establishment and maintenance of epigenetic modification greatly differs among them. Mammals have a clear reprogramming and maintenance mechanism for epigenetic state, featuring erasure and reestablishment of molecular markers. In contrast, plants possess a reversible mechanism, in which methylation and demethylation enzymes appear to be important. Moreover, what is critical in plants is the chromatin structure, which changes in response to environmental stimuli. The detailed molecular mechanisms, however, await further investigation. Analyses of links among DNA methylation, histone modification and chromatin structure will necessary to decipher the processes involved.

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