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RESEARCH ARTICLE

INSIGHT INTO THE DNA METHYLTRANSFERASES IN PLANTS AND ANIMALS

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ABSTRACT

DNA methylation is the most common epigenetic phenomenon that controls gene regulation. In animals, it has been implicated in a number of biological phenomena, including genomic imprinting, X- chromosome inactivation, tissue specific gene expression, silencing transposable elements and regulation of gene expression. In plants the function of DNA methylation is well known in silencing of transgenes, transposons and pseudogenes. DNA methylation has also been characterized by self-incompatibility and maternal inheritance. Alteration in DNA methylation is associated with various human diseases. DNA methylation plays fundamental roles in the regulation of gene expression and is essential for plant and animal development. In animals, Dnmt1, Dnmt3a and Dnmt3b maintain the methylation whereas plants have three classes of DNA methyltransferases that maintain the methylation pattern. In this review, we discuss the role of mammalian and plant DNA methyltransferases, focusing on their structural and functional features as well as their roles in gene regulation.

Key words: DNA methylation, Dnmt1, Dnmt3, MET1, DRM2, CMT3, RNA directed DNA Methylation.

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INTRODUCTION

The discovery of DNA in the 20th century is a landmark finding in molecular biology research. It is in the form of four functional nitrogenous bases (A, T, G and C) that encode the molecular message of life. Out of the four nitrogenous bases, cytosine and adenine are usually found in methylated forms. The methyl group on the 5th carbon of cytosine is so plentiful that it is called as the 5th base of DNA. The methylation in cytosine is frequently found on CpG islands in animals, but in case of plants in addition to CpG, CpHpG and CpHpH sites are also the hot spots for cytosine methylation. Jhonson and Coghil (1925) first reported 5-methylcytosine as a constituent of nucleic acid. Later on Wyatt (1950) reported its existence in plants. The presence of methyl cytosine in plants and animals indicates that this must have an important role in gene regulation. With the advancement of science, when the transcriptome and methylome data were analyzed, it was found that DNA methylation in most of the cases is inversely related to the rate of transcription. So cytosine methylation is a part of gene silencing machinery. Intensive studies on DNA methylation reveal that it controls X chromosome inactivation, silences transposons, psuedogenes, repeat elements, imprinted genes and is also responsible for self-incompatibility in plants. Recent studies on genome wide methylation indicate that pattern and amount of DNA methylation regulate the expression of a gene. Interestingly, the methylation pattern is inherited from parents to progeny.

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Gene body methylation and Promoter DNA methylation

The functional unit of a gene contains two parts, the promoter and the body. The promoter drives the expression of the gene, whereas the body contains the coding part that makes a functional protein. Higher eukaryotes show a biased methylation pattern in these two parts. DNA methylation in the promoter region inhibits the binding of transcription factor to the *cis* acting element and regulates the gene expression. The methylation of promoters is associated with tissue specific expression (Zhang *et al.,* 2006). DNA methylation in the coding region of the gene is called gene body methylation. Although the function of gene body methylation is not yet clear; it is still believed that it has an important role in gene regulation, including splicing and preventing aberrant expression of the gene by intergenic promoters (Takuno and Gaut 2011). Most of the body methylated genes encode for catalytic enzymes (Zhang *et al.,* 2006). This suggests that plants have evolved to regulate the expression of catalytic enzymes at the level of the epigenome. The mammalian genes contain small transposons and repetitive elements within the coding region which are usually methylated. In contrast plants do not contain such elements, but still they show a high degree of cytosine methylation in the coding region. About one third of the genes in *Arabidopsis* show high level of DNA methylation in the gene body as compared to the promoter

(Zhang *et al.,* 2006). Another interesting fact is that the 3' and 5' regions of the constitutively expressed genes are hypo-methylated, suggesting the role of DNA methylation in transcriptional initiation and termination (Tran *et al.,* 2005; Zhang *et al.,* 2006). The body methylated genes are larger in size as compared to the unmethylated genes and are functionally more important (Takuno and Gaut *et al.,* 2011). DNA methylation in the promoter region regulates the expression of the gene. Many transcription factors bind to *cis* acting elements in the promoter, but due to methylation in promoter region the transcription factors are unable to bind to these the elements. The promoter methylated genes show tissue specific activation (Zhang *et al.,* 2006). Hypermethylation of the promoters represses the gene expression whereas hypomethylation leads to activation. In *Arabidopsis* hypermethylation in the promoters of the *MAPK12*, *GSTU10* and *BXL1* genes in callus cells leads to silencing (Berdasco *et al.,* 2008). Promoter methylation is *MET1* and *DRM2* dependent as it usually occurs at CpG sites (Berdasco *et al.,* 2008). In mammals the proximal region of the genes encoding Trefoil Factor 1 (TFF1) and Estrogen Receptor a (ERa) could be partially methylated by treatment with deacetylase inhibitors, suggesting the possibility of dynamic changes in DNA methylation. These promoters show cyclic methylation and demethylation of CpG dinucleotides which regulate their expression (Kangaspeska *et al* 2008). In maize, the promoter of phoshoenolpyruvate carboxylase (PEPC) gene is hypermethylated in roots and light induces demethylation of this promoter in mesophyll cells (Tolley *et al.,* 2011).

DNA methyltransferases in animals

DNA methylation plays an important role in epigenetic signaling, especially in the regulation of gene expression by modulating the dynamics of chromatin structure. In animals, DNA methylation occurs at the 5th carbon of cytosine residue in CpG islands. In mammals, three DNA methyltransferases are present, named as Dnmt1, Dnmt3a and Dnmt3b. In addition to these, another protein without catalytic activity, called Dnmt3L is also present. The DNA methyltransferases in mammals contain a large multi-domain N-terminal part having a regulatory function, and a C-terminal catalytic part (Figure1). The N-terminal part is essential for nuclear localization of these enzymes and mediates their interactions with other proteins, DNA and chromatin. The smaller C-terminal part contains the active site of the enzyme and ten amino acid motif conserved for all DNA methyltransferases (Jeltsch *et al.,* 2002). The catalytic domains of all DNA methyltransferases share a common structure, called "AdoMet-dependent methyltransferase fold" (Cheng *et al.,* 2008). Besides the conservation in the structure, DNA methyltransferases share an important mechanistic similarity; they all flip their target base out of the DNA helix and bury it in a hydrophobic pocket of the active site. The catalytic mechanism of C5 DNA methyltransferases involves the nucleophilic attack of the enzyme on the sixth position of the cytosine by the catalytic cysteine residue located in motif-IV (PCQ motif), which leads to the formation of a covalent bond between the enzyme and the substrate base. This reaction increases the negative charge density at the C5 atom of the cytosine, which attacks the methyl group bound to AdoMet.

Dnmt1 (DNA methyltransferase1)

Dnmt1 is the well characterized enzyme of mammalian systems. It shows a preference for hemimethylated DNA over unmethylated DNA and is localized at DNA replication foci during the S phase (Goyal *et al.,* 2006). This suggests that it is a maintenance methyltransferase. It is constitutively expressed in proliferating cells and is the major DNA methyltransferase in somatic tissues (Robertson *et al.,* 2000). The expression of *Dnmt1* varies in a cell-cycle-dependent manner. It expresses maximum during the S phase (Kimura *et al.,* 2003). It is also regulated by post-transcriptional processes (Torrisani *et al.,* 2007). Dnmt1 is distributed throughout the nucleus during interphase, whereas in the early and mid S-phase, it gets localize to the replication foci in cells, actively synthesizing DNA. Different isoforms of Dnmt1 have been identified. Besides Dnmt1, which is present in most somatic cells, two tissue-specific Dnmt1 variants called Dnmt1o (Dnmt1 oocyte) and Dnmt1p (Dnmt1 pachytene), have been described in oocytes and spermatocytes, respectively (Mertineit *et al.,* 1998). Dnmt1o is an oocyte-specific splicing isoform of Dnmt1 that lacks the first N-terminal domain. This isoform shows higher stability than Dnmt1, which could explain its use during oocyte growth and maturation (Ding *et al.,* 2002). It can functionally replace Dnmt1 in somatic cells, because mice expressing Dnmt1o instead of Dnmt1 in all somatic tissues are phenotypically normal (Ding *et al.,* 2002). Interestingly, Dnmt1o is localized to the cytoplasm during the preimplantation development of the embryo, except in the eight-cell stage, in which it was reported to be transiently translocated to the nucleus (Carlson *et al.,* 1992; Ratnam *et al.,* 2002). Dnmt1 shows a preference for hemimethylated DNA over unmethylated substrate, supporting its role as a maintenance methyltransferase (Goyal *et al.,* 2006). Its intrinsic preference for hemimethylated DNA has been estimated to be about 30-40 fold (Jeltsch *et al.,* 2006).

The preference for hemimethylated sites is due to an interaction of hemimethylated CG sites with the active center of the enzyme, followed by methylation-specific conformational changes of the enzyme, leading to the activation of the enzyme. The detailed molecular mechanism of the recognition of a methylated cytosine in the non-target DNA strand of the CG site is not known. Dnmt1 is responsible for the re-establishment of DNA methylation after DNA replication. The enzyme occupies a position at the replication fork, where it works as a molecular copy machine, and quickly methylates the hemimethylated CG dinucleotides, thereby restoring the original methylation pattern. It is a high fidelity enzyme, able to methylate long stretches of DNA without dissociation (Vilkaitis *et al.,* 2005; Goyal *et al.,* 2006). Interestingly, methylation is possible only in one strand of the DNA; Dnmt1 does not swap its target strand while moving along its substrate. These properties prepare the enzyme to follow DNA replication and to methylate the new DNA strand before the chromatin is reassembled. In addition to its well-known role as a maintenance DNA methyltransferase, Dnmt1 is also required for *de novo* DNA methylation. It shows allosteric regulation. The binding of unmethylated DNA at N-terminal region reduces the activity of the enzyme whereas binding of methylated DNA increases its activity (Pradhan and Esteve2003; Svedruzic and Reich 2005). This suggests that the methylated regions of the genome tend to be more methylated and the unmethylated regions lose methylation (Eckhardt *et al.,* 2006). Another study suggests that the phosphorylation at the 515th serine of N-terminal domain enhances the catalytic activity of the enzyme by an allosteric process (Goyal *et al.,* 2007). Dnmt1 is an important part of the epigenetic network as it interacts with many components of the silencing machinery,

such as the histone deacetylases (HDAC1 and HDAC2), DNA methyltransferases (Dnmt3a, and Dnmt3b), methyl CpG binding domain proteins (MeCP2), transcription factors (Rb and E2F1), histone lysine methyltransferases (Suv39H1, the SET7/9, G9a and EZH2) and heterochromatin protein1 (HP1) (Fuks *et al.*2000; 2003 Robertson *et al.,* 2000, Rountree *et al.,* 2000, Kim *et al.,* 2002, Kimura *et al.,* 2003, Esteve *et al.,* 2006, Vire *et al.,* 2006, Bostick *et al.*2007, Sharif *et al.,* 2007, Jung *et al.,* 2007). Recent studies identified itsinvolvement inimpairment of wound healing intype 2 diabetes by Dnmt1-dependent regulation of hematopoietic stem cells (Yan et al 2018).

Dnmt 3 (DNA methyltransferase3)

The mammalian *de novo* methyltransferase Dnmt3, family comprises three members: Dnmt3a, Dnmt3b and Dnmt3L. The first two are mostly responsible for the establishment of DNA methylation. Dnmt3L is catalytically inactive and functions as a regulatory factor in germ cells. Both Dnmt3a and Dnmt3b do not display any significant preference between hemimethylated and unmethylated DNA (Okano *et al.,* 1998; Gowher *et al.,* 2001). However there are some reports that they also play a role in the maintenance of DNA methylation at heterochromatic regions (Kim *et al.,* 2002, Liang *et al.,* 2002, Chen *et al.*2003; Jeong *et al.,* 2009). Like other methyltransferases the Dnmt3 enzymes also possess a N-terminal regulatory part and a C-terminal catalytic part harboring the conserved C5 DNA MTases motifs. Dnmt3a interacts with many proteins, such as the transcription factors PU.1, Myc, RP58, histone deactetylase HDAC1, heterochromatin protein HP1, histone methyltransferases, SUV39H1, SETDB1, EZH2, methyl CG binding protein MBD3 and chromatin remodelling factor Brg1 (Fuks *et al.,* 2001; Suzuki *et al.,* 2006, 2006; Li *et al.*2006; Vire *et al.,* 2006). Dnmt3a, Dnmt3b and Dnmt3L have recently been shown to interact specifically with the N-terminal part of histone H3 tails unmodified at lysine 4, the binding being disrupted by the methylation of H3 at K4 residue (Ooi *et al.,* 2007; Otani *et al.,* 2009). Although Dnmt3a and Dnmt3b methylate cytosine residues predominantly in the CG context, but there are reports that both enzymes can also modify cytosines in a non-CG context (Ramsahoye *et al.,* 2000; Gowher and Jeltsch 2001).

The methylated non-CG sites are found in ES cells (Embryonic Stem cells), where Dnmt3a and Dnmt3b enzymes are highly expressed, but not in the fetal lung fibroblasts or monocytes, where Dnmt3 enzymes are down-regulated (Lister *et al.,* 2009; Laurent *et al.,* 2010). Dnmt3a and Dnmt3b show strong preferences for methylation of CG sites embedded into different flanking sequences. Dnmt3a and Dnmt3b prefer methylation at CpG sites when the flanking region contain purine bases at the 5'-end of the CG site, whereas pyrimidines were favored at its 3'-end (Handa and Jeltsch 2005). Dnmt3a gene polymorphism contributes to daily life stress susceptibility and mutation of Dnmt3a leads to genome instability (Barliana et al 2017: Banaszak et al 2017).Mutations in the human Dnmt3b gene cause an autosomal disease, called ICF (Immunodeficiency, Centromere instability, Facial abnormalities) syndrome (Okano *et al.,* 1999, Hansen *et al.,* 1999). Mutations in Dnmt3b protein result either in the reduction of the catalytic efficiency of the enzyme or in alteration of its localization, causing specific loss of DNA methylation at satellites 2 and 3 of the pericentromeric regions of chromosomes 1, 9 and 16. The hypomethylation of these regions leads to recombination events, resulting in chromosomal rearrangements. Dnmt3L knockout transgenic mice do not show discernible morphological abnormalities (Hata *et al.,* 2002). However, male mice lacking Dnmt3L are sterile, because they fail to produce mature sperms, whereas male germ cells lacking Dnmt3L show reactivation of retrotransposons of the LINE-1 (long interspersed nuclear element 1) and IAP (Intracisternal A particles) classes, severe defects in meiosis, which result in the loss of all germ cells (Bourc'his and Bestor 2004; Webster *et al.,* 2005).

The Dnmt3L knockout phenotype in female mice is different because females are fertile, but fail to deliver viable pups and the developing embryos die as a result of defects in the development of the neural tubes. Loss of Dnmt3L leads to specific hypomethylation of maternally imprinted genes (Bourc'his *et al.,* 2001; Hata *et al.*2002). Dnmt3a, together with Dnmt3L is required for the proper establishment of imprinting during gametogenesis. Both enzymes are involved in the methylation of different subsets of repeat elements. Dnmt3b is responsible for the methylation of pericentromeric minor satellite repeats. Constitutive or conditional deletion of Dnmt3b, but not Dnmt3a, in mouse embryonic fibroblast (MEF) cells results in partial loss of DNA methylation throughout the genome. Demethylation leads to genomic as well as chromosomal instability (Dodge *et al.,* 2005). Dnmt3a and Dnmt3b are highly expressed in embryonic tissues and undifferentiated ES cells and down-regulated in differentiated cells. Both Dnmt3a and Dnmt3b are stably associated with chromatin containing methylated DNA (Jeong *et al.,* 2009), including mitotic chromosomes, and localize to pericentromeric heterochromatin (Chen *et al.,* 2004; Ge *et al.,* 2004). The nuclear and subnuclear localization of Dnmt3L depends on its interaction with Dnmt3a or Dnmt3b. In the absence of Dnmt3a and Dnmt3b, Dnmt3L is distributed diffusely throughout the nucleus and cytoplasm, but after binding to Dnmt3a it concentrates in chromatin foci (Nimura *et al.,* 2006). Sumoylation of Dnmt3a and Dnmt3b has been reported in the N-terminal domains of the enzymes. Sumoylation of Dnmt3a disrupts its ability to interact with histone deacetylases (Kang *et al* 2001, Ling *et al* 2004; Li *et al* 2007).

Enzymes involved in DNA methylation in plants

The methyltransferases in plants can be broadly divided into two major classes, the *de novo* methyltransferase and maintenance methyl transferases. Methylation of fully unmethylated DNA is called *de novo* methylation whereas methylation of hemi methylated DNA, produced after DNA replication is called maintenance methylation. Both the types are essential for plant development and successful completion of its life cycle. The methyltransferases discovered so far have two domains, the N terminal regulatory domain and the catalytic C terminal domain. Theplant methyltransferases belong to four families according to their domain arrangement namely MET, DRM, CMT and Dnmt2. The MET and CMT are the maintainance methyltransferases whereas DRM is a *de novo* methyl transferase.

DRM (Domain Rearranged Methyl transferase)

Domain Rearranged Methyltransferase (DRM) enzymes are responsible for *de novo* DNA methylation of unmethylated

DNA. This class of enzymes is present only in plants, although they show homology to mammalian DNMT3 (Cao *et al.,* 2000). The DRM enzymes differ from Dnmt in having a unique N-terminus containing an ubiquitin associated domain (UBA). In the model plant *Arabidopsis* there are three DRM enzymes present (DRM1, DRM2 and DRM3). Studies on the mutants of DRM1 and DRM2 suggest that these two enzymes are responsible for cytosine methylation in CHG and CHH sites and have lesser roles in CG methylation(Cao and Jacobsen 2002). Tobacco DRM methylates CHG and CHH sites with very less activity towards CG sites. This enzyme prefers unmethylated DNA rather hemimethylated DNA (Wada *et al.,* 2003). The DRM1 and DRM2 double mutant do not loose methylation pattern, but after many generation they block CHG methylation. The phenotype of the double mutant is similar to the mutants of small RNA-related genes, which suggest that DRM may be involved in RNA directed DNA methylation (Cao *et al.,* 2003; Chen et al.2004; Zilberman *et al.,* 2004). AtDRM2 is also involved in nucleolar dominance and rRNA gene silencing through RdDM (Preuss *et al.,* 2008). DRM2 interacts with RDM1 (RNA directed DNA Methylation1) and AGO4 suggesting its involvement in RdDM (Gao et al 2010; Zhong et al 2014). The dimerization of DRM2 is required for its catalytic activity. In rice and Arabidopsis DRM2 interacts with ATP dependent RNA helicase OseIF4A through ubiquitin-associated domain (Dangwal et al 2013). These two proteins associated at the target sites for RdDM.

MET1 (methyl transferase1)

In plants the major maintenance methyltransferase is MET1 (methyltransferase 1), which shows homology with mouse Dnmt1 (Finnegan and Dennis 1993). This protein has a large N terminal regulatory domain and a NLS signal. MET1 also contains a bromo adjacent homology (BAH) domain, which is supposed to be involved in protein-protein interaction (Callebaut *et al.,* 1999). In *Arabidopsis* there are four members present in MET family (METI, METIIA, METIIB and METIII) (Genger *et al.,* 1999). The *met1* mutant of *Arabidopsis* shows abnormal phenotype, like late flowering and reduced fertility (Kankel *et al.,* 2003; Saze *et al.,* 2003). The mutant plant also shows reduction in CG methylation in the genome (Kankel *et al.,* 2003). Global transcript level in *met1* mutant shows increased level of transcription in both methylated and unmethylated genes (Zilberman *et al.,* 2007), and few genes show misexpression such as IBM1 (Saze *et al.,* 2008), sadhu6-1 and RPS (Singh et al.2008). During gametogenesis MET1 maintains the methylation status. Loss of MET1 leads to passive DNA demethylation (Saze *et al.,* 2003). MET1 is also important for maintaining imprinted genes like FWA and MEDEA (Xiao *et al.,* 2003, Kinoshita *et al.,* 2004; Pien *et al.,* 2007). MET1 interacts with histone deacetylase6 through the BAH domain and regulate transposone silencing (Liu et al 2012). MET1 interacts with MEA1, a histone methyltransferase and is part of Fertilization-Independent Seed Polycomb Repressive Complex 2 (FIS-PRC2) and is involve in repression of autonomous endosperm development (Schmidt et al 2013).

CMT (chromo methyl transferase)

This class of methyltransferases is unique to plants and its members mainly control non CG methylation (Cao and Jacobsen 2002). These enzymes contain a conserved region of about 60 amino acids residues called chromodomain in between II and IV motifs. The regulatory region contain a BAH domain which is similar to other methyltransferases. The chromodomain is also found in some heterochromatin proteins and polycomb group of proteins. This suggests that these enzymes have role in methylating DNA in heterochromatin region of the genome (Eissenberg *et al.,* 2001; Papa *et al.*2001). In *Arabidopsis* three genes represent this class of enzymes (*CMT1*, *CMT2*, and *CMT3*). Mutation in *CMT3* locus leads to loss of global non-CG methylation at repetitive centromeric region and activation of transposon. The mutant also shows decreased methylation of SUPERMAN and Phosphoribosyl Anthranilate Isomerase (PAI) loci in *Arabidopsis* (Lindroth *et al.,* 2001; Bartee *et al.,* 2001). CMT is also essential for the silencing of retroposon like Ta3 (Lindroth *et al.,* 2001; Tompa *et al.,* 2002). CMT3 is regulated by post transcriptional modifications. Two E3 ligase, a ubiquitin ligase, JMJ24 and a SUMO ligase, SIZ1 interacts with CMT3 (Kim et al 2015; Deng et al 2015). The SUMOlyzation enhances the activity of CMT3 whereas ubiquitination triggers proteasomeal degradation of CMT3.

Conclusion and future prospect

DNA methylation is an important epigenetic maker and an integral part of the gene regulation. DNA methylation plays essential role in tissue and organ differentiation by regulating stage specific gene expression. Recent technical advances such as whole-genome bisulphite sequencing generated large-scale data for epigenetic modifications and extended our view to a genome-wide scale. This review provides important insight for diverse fields such ashuman health, plant development and evolution.

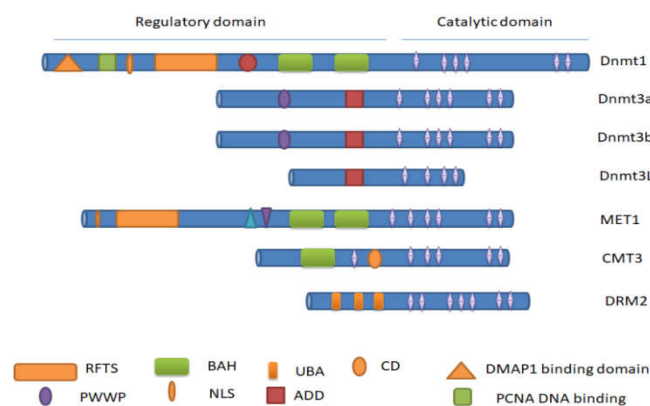

Figure 1. Domain organization of DNA methyltransferases

Domain architecture of plant and mammalian DNA methyltransferases showing the catalytic and regulatory domain.. The different domains are indicated by different colored boxes, indicated in the below.

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