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REVIEW ARTICLE

DNA Methylation: A Stabilizing and Regulatory Mechanism of Plant Genome

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ABSTRACT

The immobile lifestyle of plants requires responses to adapt the environmental stress. Flexible epigenetic regulations are essential for reprogramming of plant gene expression. The overall phenotype and gene expression profile of an organism is controlled by mechanisms other than the normal mechanism of expression. DNA methylation is one of them which control many important cellular functions, such as transposon silencing, genome stability, cell identity maintenance and defense against exogenous DNAs. DNA methylation maintained by a set of enzymes named DNA (cytosine-5-)-methyltransferases (DCMTases). In this paper, types, importance, mechanism, maintenance, and impact of DNA methylation on plant genome expression and transposition have been discussed. Methods to detect DNA methylation and CpG islands in plants genome has also been explored.

Key words: Transposon; CpG iselands; Methyltransferases; Transcriptional; posttranscriptional

1) INTRODUCTION

The lifestyle of plants requires increased developmental plasticity in which flexible epigenetic regulation is essential for reprogramming of gene expression for rapid responses and adaptation to environmental cues. Closely related species having differences in gene expression in response to environmental stresses shows differences in their epigenetic system [1]. The recent work suggested that abiotic and biotic stresses including DNA damage, drought, high salinity and pathogens are sources of epigenetic variation [2]. The covalent modifications of DNA, RNA, and chromatin structure are basic components of genetic and epigenetic regulation of genome expression. They are also regulated by an array of proteins or protein complexes, leading to specific profiles of chromatin modification and remodeling. Methylation, a direct chemical modification occurs at a defined target sequence at each step of the central dogma. In mammalian DNA methylation occurs throughout the genome, while in plants DNA methylation predominates at transposons, other repeat sequences and centromeric regions [3]. Plant genomes are generally more methylated compared to other eukaryotic genomes. This is not only due to extra CG dinucleotide sequences in plants but also to methylation of cytosine in the trinucleotide CpNpG and CpNpN sequence [4]. It is called the CpG islands (CGIs) where "p" simply indicates that "C" and "G" are connected by a phosphodiester bond. The CGIs are short stretches (200 to 3000 bp) of DNA in which the frequency of the CG sequence is higher (60%) than other regions. CGIs often located around the promoters of about 70% of known active genes (Actually transcriptional promoters) have enhancer elements interdigitated between and generally not methylated at these locations with few exceptions (e.g., differentially methylated regions associated with gene imprinting) but methylation could occur when finding in exons, transposable elements and satellite DNA [5].

DNA methylation blocks gene transcription when present in promoter regions [6]. However, methylation is also known to occur in plant RNAs but the impact of these modifications on gene expression regulation has to be explored [7-8]. Recent papers suggested that RNA methylation is an essential negative regulator of gene expression in mammalian cells and increased the frequency of alternative splicing [9-10]. In plant RNAs adenosine of the trinucleotide GAC and AAC, with a 75% preference for GAC, Methylated at N6 position of ring and form N 6-methyladenosine [11]. It is a ubiquitous base modification found internally in the mRNA of various classes including ribosomal RNAs, small nuclear RNAs, and transfer RNAs [12]. The trinucleotide GAC and AAC where methylation takes place called m6A which is predominantly positioned 100–150 bp before the poly(A) tail toward the 3 end of transcripts [11].

2) MECHANISM OF DNA METHYLATION

Methylation of DNA is catalyzed by a set of enzymes

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named DNA (cytosine-5-)-methyltransferase (DCMTases). Plants have four classes of DCMTases namely DNA methyltransferase1 (MET1), domains rearranged methyltransferase (DRM), DNA nucleotide methyltransferase2 (DNMT2) and chromomethylase3 (CMT3) [1]. In plants, MET1 transfer a methyl group from S-adenosylmethionine to cytosine residues at 5th carbon position. MET1 enzyme is an ortholog of DNA methyltransferases (DNMT) enzyme present in animals, responsible for maintaining CpG methylation during DNA replication and also play a role in de novo methylation [2, 13-14]. The symmetric CpNpG methylated by a plant-specific DNA methyltransferase i.e. chromomethylase3 (CMT3) [3, 15-16]. The asymmetric CpNpN methylation is maintained by domains rearranged methyltransferase2 (DRM2), a homolog of DNMT3A/DNMT3L enzyme in animals [17]. Methylation at a CpNpN locus will be lost in one daughter DNA strand. DRM2 is an enzyme required for de-novo methylation of all three sequence contexts CpG, CpNpG, CpNpN and which dependent on RNAi-like machinery [1, 18-20]. Plant genomes also encode a catalytically inactive methyltransferase domains rearranged methyltransferase3 (DRM3). DRM3 controls DNA methylation through its functional interaction with a plant-specific RNA polymerase V (Pol V) and through regulation of Pol V-dependent noncoding RNA transcription [3, 17, 21]. The DNA methylation in plants is guided by small noncoding RNAs (snRNAs) results in transcriptional gene silencing (TGS) and the process is known as RNA-directed DNA methylation (RdDM) [15-16, 22]. Two plant-specific RNA polymerases, Pol IV and Pol V act at different steps of RdDM pathway, Pol IV required for 24-nucleotide (nt) siRNA biogenesis and Pol V functioning as a downstream effector for DNA methylation [23].

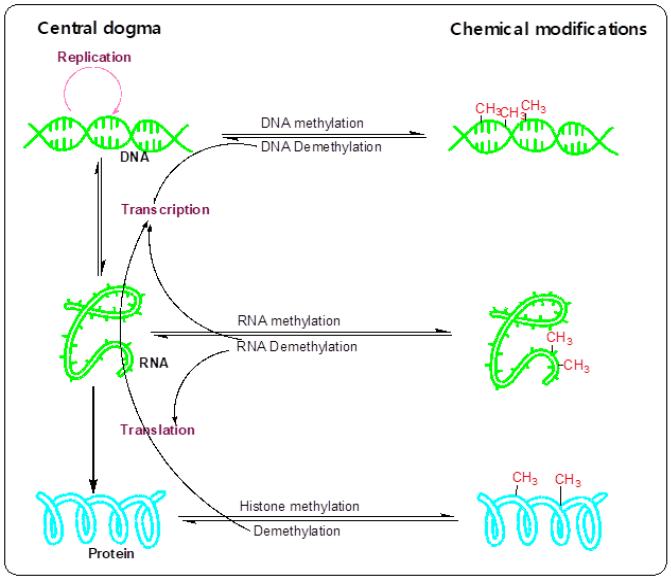

Figure-1: Methylation at different steps of central dogma of life and their impact on different gene regulation processes.

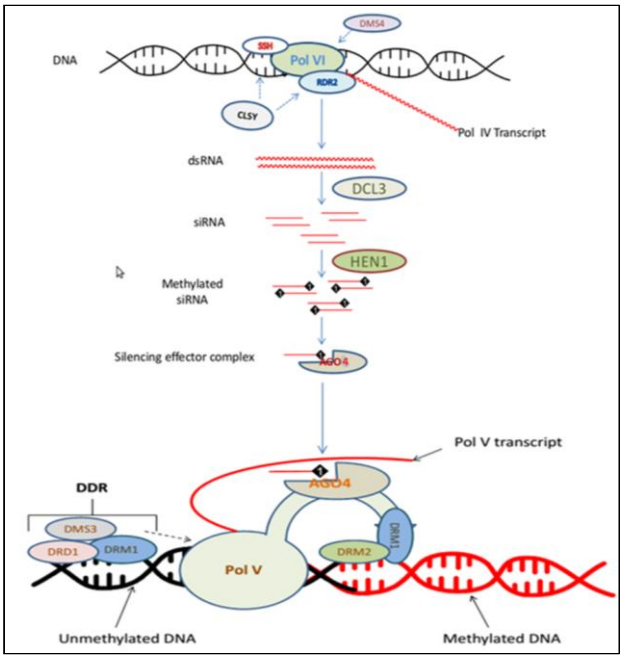

Figure- 2: Steps of molecular mechanism of RNA directed DNA methylation.

During RdDM 21-nt long sRNAs establish DNA methylation, whereas the 24-nt long sRNAs are responsible for its amplification and maintenance [6]. microRNAs, ta-siRNAs, 21 nt siRNAs induced DNA methylation also takes place by non-canonical RdDM mechanisms which often needed the protein factors involved in post-transcriptional gene silencing (PTGS) besides this some components are also essential for canonical RdDM [14, 24]. Pol IV transcribed transposons and repeat loci with the assistance of the SNF2-like putative chromatin remodeling protein classy1 (CLSY1) and the homeodomain transcription factor-like DNA-binding transcription factor 1/Sawadee homeodomain homolog 1 (DTF1/ SHH1) [25-29]. RNA-Dependent RNA polymerase2 (RDR2) copied the resulted transcripts into double-stranded RNAs (dsRNAs) than the resulted dsRNA processed into 24-nt siRNA duplexes by dicer-like 3 (DCL3) [30-31]. Subsequently 3'ends of the siRNAs methylated by RNA methylase HEN1 for stability and the single-stranded siRNAs loaded into argonaute (AGO4) [32-34]. Base-pairing between the siRNA and nascent transcript leads Pol V to recruit siRNA-bound AGO4 [35]. A putative chromatin remodelling complex termed DDR (consisted of defective in RNA-directed DNA methylation 1 (DRD1), defective in meristem silencing 3 (DMS3) and domains rearranged methyltransferases 1 (RDM1)) also required for Pol V association with chromatin and transcription [21, 23, 36]. The association of RDM1 protein of DDR complex with AGO4 and DRM2 may help to recruit DRM2 to catalyze DNA methylation Pol V-target regions [23, 36-37]. DNA methylation initiated and reinforced in plant embryos by siRNAs produced in the vegetative cell of pollen and the central cell of endosperm but didn't contribute genetic material to subsequent generations [38-39].

3) PURPOSE OF DNA METHYLATION

Known purpose of DNA methylation is not clear till date but the best hypothesis we have is as follows:
1. RNA polymerase starts randomly transcribing DNA. Transcription factors just increase transcription at genes, but that is non-specific genome-wide transcription happening.
2. The nonspecific transcription is wastage of cellular energy which has finite resources. If random DNA is transcribed it causes down-regulation of a gene by RNAi pathways, which are potentially harmful, when not supposed to.
Thus, DNA methylation helps to prevent deleterious consequences by preventing transcription where it has no business, however, non-specific transcription still happens but probably at a much lower rate.

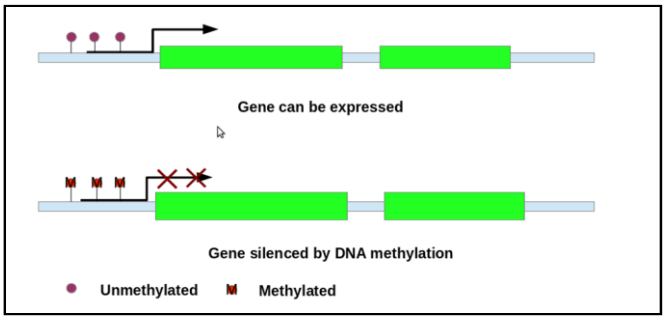

Figure-3: Regulation of gene expression by methylation of promoter regions.

4) TYPES OF DNA METHYLATION

DNA methylation is intergenic and intragenic but the role remains unclear. One role for intragenic methylation may be in regulating cell context-specific alternative promoters in gene bodies [40] and perhaps also prevent aberrant expression from intragenic promoters. Another possible reason for intragenic methylation is its capability to increase the accuracy of splicing. The purpose of methylation at CpG islands would be primarily to silence or downregulate gene expression. This silencing may be two types: the first one is permanent where genome globally methylated (during macro and microsporogenesis) and other is scenarios where genome cyclically methylated and demethylated at specific gene loci [41]. Once DNA methylation happened the methylated portion of DNA wrapped up around histones for completing the silencing of the gene. In contrast to the silencing role of methylation in promoter regions, methylation of gene bodies may promote expression potential. Both passive and active demethylation naturally happens. Four bifunctional 5-methylcytosine glycosylases: Repressor of silencing 1 (ROS1), Demeter (DME), DME-like 2 (DML2), and DML3 are responsible for DNA demethylation, which removes methylated bases by cleavage the DNA backbone at a basic site [5]. The DME are responsible for genome-wide DNA demethylation and gene imprinting in central cells and endosperms [42]. Passive DNA demethylation happens, when DNA methylation pathways are inactivated [5]. A rapid loss of paternal methylation pattern has been observed in pollen vegetative cells, endosperm during seed development, before merging of paternal and maternal genomes and early in development, a subset of cells in the developing embryo instructed to become germ cells undergoes global erasure of methylation patterns which is active demethylation [1]. The genome-wide demethylation in female gametogenesis is accompanied by extensive non-CG hypermethylation of siRNA-targeted transposon sequences [5]. In certain scenarios, at specific loci, DNA methylation and demethylation appear to be even a cyclic process [41].

5) MAINTENANCE OF DNA METHYLATION

The beauty of this silencing process is that, once a gene is silenced, by methylation, this state is faithfully propagated with every cell division, preserving cell state by methylated of the template DNA strand just after replication. The CpNpG methylation maintained by DNA methyltransferase named chromo methylase 3 (CMT3) while methylation at a CpNpN locus will be lost in one daughter DNA molecule after replication [42]. The initiation of methylation and active removal of methylation both controlled by siRNAs, transcription factors in addition to other factors [44]. 24-nucleotide small interfering RNAs (24-nt siRNAs) and long non-coding RNAs (lncRNAs) direct de novo DNA methylation and transcriptional gene silencing [22]. The methylated cytosine may be converted to thymine by accidental deamination. Unlike the cytosine to uracil mutation which is efficiently repaired, the cytosine to thymine mutation can be corrected only by the mismatch repair which is very inefficient. Hence, over evolutionary time scales, the methylated CG sequence will be converted to the TG sequence. This explains the deficiency of the CG sequence in inactive DNA portion. The methyl group of a CpG is positioned in the major groove of the DNA and binds with methyl CpG-binding proteins (MBD1, MBD2, and MeCP2). Most transcription factors make heavy use of the major groove to read the bases. A methyl group and methyl CpG-binding proteins here can make the enhancer unrecognizable.

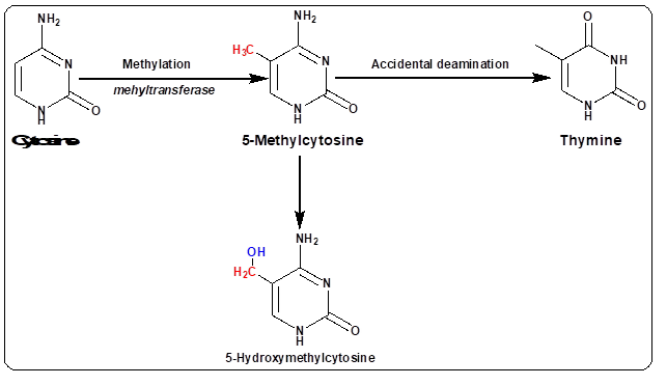

Figure-4: Methylation of cytosine at 5th carbon, conversion of Cytosine into Thymine by accidental deamination of 5-Methyle Cytosine and formation of 5-Hydroxymethylcytosine by hydroxylation of CH_3 group of 5-Methyle cytosine.

6) IMPACT OF METHYLATION

Plants are well known for having transposon dominated genomes. When transposable elements are active, all sorts of things go haywire, from DNA to gene expression, and ultimately the phenotype. The vast majority of transposable elements were inactivated by DNA methylation or by mutations acquired over time as the result of the deamination of 5mC [45]. DNA methylation silence transposable elements especially Retrotransposons (active transposons) and another DNA repeat to promote genome stability [46]. Besides transposable element silencing, DNA methylation also plays critical roles in diverse aspects of biological processes, including genome integrity, imprinting, development, X chromosome inactivation, retrovirus suppression, and stress responses [14, 47-50]. Once established, DNA methylation is epigenetically inherited [51]; thus, demethylated TEs may eventually be silenced making them more available for exaptation [52]. De silence TEs allows the production of small RNAs that can silence transposons in more critical cells by RdDM pathway [5]. 21–24 nt long small RNAs (sRNAs) direct the RNA silencing machinery to target nucleic acids in a sequence-specific manner [6]. Epigenetic regulation of TEs can alter the expression of nearby ordinary genes, and cis-regulatory elements exaptation [46, 53-54]. Global epigenetic de-silencing increases the rate of transposition, so it may enable periods of rapid evolution and ultimately punctuated equilibrium [55-59].

In different species, the amount of CpG methylation is directly proportional to the amount of noncoding DNA (transposons). A change in DNA methylation pattern during major developmental phases is a good evidence to suggest that CpG methylation has a genome-wide control for gene expression [60]. During early embryogenesis, CpG islands in front of promoters appear to be primarily protected from methylation the possible reasons may be the island shape or transcription factor binding [61]. There is evidence that proteins bind CpG islands and deny access to the MET. The CpG islands remain unable to retain their unmethylated state when transcription factor binding sites mutated [62-63]. Thus this mechanism enables the turning off a gene to happen at the right time during the developmental process. One interesting observation in a paper of Takuno and Gaut was that in A. Thaliana, methylated genes were evolved slower than unmethylated genes despite the potential for increased mutations in methylated CpG nucleotides [64]. This is due to a higher selective constraint on them. Gene-body methylation also plays a role in tissue-specific gene expression based on an observation that constitutively expressed genes tend to have more methylation than tissue-specifically expressed genes [3]. For the better understanding of the impact of DNA methylation see the review article of Kohli and Zhang on the topic of DNA demethylation [65] and another excellent review on methylation titled DNA methylation: role in mammalian development [66]. The RNAi system itself may be an indirect exaptation of TE-genome coevolution and perhaps originally evolved to regulate TEs [67]. Epigenetic regulation is now used for various purposes such as genomic imprinting [68], gene body methylation [69], developmental plasticity, and the buffering of developmental programs [70-74]. A huge change in methylation during each step of seed development, floral development and a transition from juvenile to adult stages takes place. DNA demethylation has also been observed in post-mitotic cells or at specific loci in rapid response to environmental stimuli indicating active demethylation happens in various biological settings [1].

7) DETECTION OF DNA METHYLATION

DNA methylation detection techniques could be divided into four groups: chemical modification with bisulfite (represented by bisulfite genomic sequencing), restriction enzyme digestion (represented by methylation-sensitive restriction endonucleases), affinity-based isolation of methylated DNA (represented by methylated DNA immunoprecipitation) and Insilico DNA methylation (Computational tools).

1. **Bisulfite genomic sequencing:** In this technique, during PCR amplification unmethylated cytosines amplify as thymine, while methylated cytosines amplified as cytosine. The methylation status can be determined through subsequent analysis of PCR product [75].
2. **Methylation-sensitive restriction endonucleases:** The technique uses restriction enzymes pair (isoschizomers) most frequently HpaII and MspI, which recognize the sequence CCGG. HpaII is blocked by methylation of either of the two cytosines, whereas MspI is blocked by methylation of merely the outer cytosine [76]. Besides HpaII and MspI, McrBC (cleaves between two methylated cytosines), MspJI (cleaves methylated cytosine when it is two nucleotides away from adenine or guanine) also used [77-78].
3. **Methylated DNA immunoprecipitation:** In this procedure, genomic DNA is sonicated into fragments and immunoprecipitated with monoclonal antibodies (antibodies produced by a single clone of cells) that specifically recognize 5-methylcytidine. A magnet used to pull the complexes out of a solution and purification is performed [79].
4. **Bioinformatics tools:** A large number of bioinformatics tools are present which can provide the possible sites of DNA methylation. i.e.
   - **MethPrimer (**http://www.urogene.org/methprimer/**):** A program analyzes input sequences for the existence of CpG islands and can also design bisulfite-conversion-based Methylation PCR Primers.
   - **CgiHunter** (http://cgihunter.bioinf.mpi-inf.mpg.de/): A software tool for CpG island annotation. Unlike many other heuristic-based approaches, the CgiHunter algorithm has been proven to identify all genome regions that meet a specified criteria and results in robust and consistent CpG island annotations.
   - **CpG island Predictor Analysis Platform /CpGPAP** (http://bio.kuas.edu.tw/CpGPAP/.): A web-based application uses complementary particle swarm optimization (CPSO), a

complementary genetic algorithm (CGA), CpGPlot, CpGProD, and CpGIS.

- **CpGcluster** (http://bioinfo2.ugr.es/CpGcluster/): A fast and computationally efficient algorithm use only integer arithmetic algorithm. All predicted CpG islands (CGIs) start and end with a CpG dinucleotide, which should be appropriate for a genomic feature whose functionality is based precisely on CpG dinucleotides.
- **Cpgplot** (http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/): A part of EMBOSS package identifies CpG islands in one or more nucleotide sequences.

8) CONCLUSION

The detailed mechanism of DNA Methylation at different stages of the central dogma of life has been discussed. In summary, DNA methylation known for its relatively stable epigenetic mark that locks gene into a silenced state. Mechanisms involve in active removal are clear however it is not clear the methylation of DNA is directly responsible for these events or a secondary event of heterochromatic compaction. DNA Methylation also provides stability to plant genome through silencing of transposon and sRNAs generation [1]. snRNAs epigenetically regulate the expression of genes at transcription and posttranscriptional. Traditionally, CpG island methylation has been thought to always be involved in turning off promoters. Studies show that CpG demethylation correlates with the activation of some normally silent genes [80]. DNA methylation/demethylation also plays a major role in Somatic embryogenesis, from an embryo without egg fertilization and development of somaclonal variance during clonal propagation [1]. As a side effect of silencing, a set of ordinary genes may also be down-regulated, perhaps via a distributed set of exonized TEs targeted by the sRNAs. Thus DNA methylation being vital to healthy growth and development of plants, it also enables the expression of retroviral genes to be suppressed, along with other potentially dangerous sequences of DNA that have entered and may damage the host genome. There is also RNA methylation process have been described to be linked to gene regulatory mechanism in other organisms but not in plants, or that might be but have not been yet investigated.

Besides in vivo DNA methylation detection methods, Bioinformatics tools will become more and more important, and necessary for global studies of methylation and CpG island detection. Special effort should be put to develop new algorithms to analyze the data as evidenced the increasing complexity of genome regulation by DNA and RNA methylation. This would help to better understand the effect of methylation on genome regulation not for plant biology alone but for the whole life science.

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