

DNA Methylation and Plants Response to Biotic and Abiotic Stress

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Abstract

DNA methylation is a conserved epigenetic modification that regulates, stabilizes, and maintains genomic integrity. Loss of DNA methylation or aberrant patterns of DNA methylation causes abnormalities in the gene regulation of plants. DNA methylation in plants is regulated by the combined action of de novo methylation, maintenance of methylation, and demethylation. The enzymes that regulate DNA methylation in plants are different but have some homology to that of mammalian DNA methylation enzymes. DNA methylation helps to develop adaptation mechanisms towards various biotic and abiotic stresses. This paper provides a comprehensive review of the DNA methylation pathway and its role in biotic and abiotic stress tolerances in plants.

Keywords: Genome, Methylation, Biotic stress, Abiotic stress, Plant

Introduction

DNA methylation is the preserved epigenetic modification regulating gene expression and stability of the genome. DNA methylation is a heritable and highly conserved epigenetic modification that doesn't alter the nucleotide sequence of the genome [1]. DNA methylation involves the covalent transfer of the methyl group to the C5 position of the cytosine base to form 5-methyl cytosine. Methyl group from SAM (S-Adenosyl Methionine) is added to the Cytosine with the help of an enzyme DNA methyltransferase [2]

Plants can contain several non-canonical base modifications in addition to the 5-methyl cytosine. These non-canonical modifications include various oxidized 5-methyl cytosine or N⁶-methyladenine (6mA) [3].

A DNA sequence is of relevance for DNA methylation. DNA methylation in plants is found in the CG, CHG, and CHH sequences context where H is A, C, or T. This pattern of DNA methylation has been observed in several plants including *Arabidopsis thaliana*, cassava, soya bean, rice, and many more crops. Methylation marks in plants are highly concentrated in heterochromatic regions, transposable elements, and repeats involving in the transcriptional gene silencing [4].

CG methylation is of importance in both animals and plants as the concentration of CG is higher in the promoter region of the genes as compared to the whole genome. CG methylation is 30 % in plants and 3.8 % in animals. There occur considerable interspecies variations within plants ranging from 4.6 % in the model plant *Arabidopsis thaliana* to 33 % in *Rey (Secale cereal)* [3,4].

Genomic DNA methylation has an important role in the evolution of the plant species, plant development, and plant physiology through the involvement in the gene expression mechanism of the plant.

There are several enzymes involved in denovo methylation and maintenance of methylation in plants. By the study in the model plant *Arabidopsis thaliana*, it was found that MET1 (DNA (cytosine-5)-methyltransferase 1) maintains CG methylation in plants, plant-specific chromomethylases (CMTs) pathways targets CHH (CMT2), and CHG sites (CMT3 and CMT1) [5]. But in the case of repeats, transposons, and asymmetrical CHH, methylation is maintained by DRM2 through persistent denovo methylation via RNA-directed DNA methylation pathway (RdDM) [6].

A recent development in the field of epigenetics has deepened our understanding of the mechanism involved in the de novo DNA methylation and maintenance of DNA methylation in plants. Recent

advancement in sequencing technologies with the ease of sequencing at a lower price has encouraged scientist to study the DNA methylation profile at the whole genomic level. In this review, we will discuss the recent progress in DNA methylation in plants, denovo methylation pathways in plants, demethylation in plants.

Materials and method

The study adopted an in-desk review of the literature. For the preparation of this review article, we followed the narrative review procedure. To avoid biases in selecting a research paper, we used the software Publish or Perish. We used keywords to search in Publish or Perish and reviewed the appeared articles.

De novo DNA methylation in plants

The phenomenon of de novo methylation in plants depends on the RNA-like mechanism via a protein called RNA-directed DNA methylation (RdDM) [6]. In plants de novo methylation in all sequences is mediated by an enzyme DOMAIN REARRANGED METHYLTRANSFERASE 2 (DRM2) [7].

RdDM involves DNA methylation of all most all cytosine residues located within a region of RNA-DNA sequence identity. RdDM is presumed to be responsible for the methylation observed in the protein-coding region of the post-transcriptionally silenced gene [7,8].

In the canonical RdDM pathway (transposons and other repeats region of the genome) aberrant single-stranded RNAs are produced by pol IV (a plant-specific version of pol II). The chromatin remodeling protein CLSY may facilitate pol IV for transcription. The single-stranded RNA is converted into double-stranded RNA by RNA-dependent RNA polymerase 2 (RDR 2) and this double-stranded RNA is cleaved into 24-nt long si RNA by a dicer-like protein known as DCL3 [8]. However, despite the identification of a large number of proteins required for the RdDM pathway, the specific mechanism of DRM2 action including its biochemical activities, interacting partners, and how DRM2 is recruited to specific loci remain largely unknown.

These siRNAs are loaded into the effector complex that contains argonaute proteins AGO4, AGO6, or AGO9. The recruitment of pol IV on chromatin depends on the histone reader SAWADEE HOMEODOMAIN HOMOLOG 1 (SHH1) and the CLASSY family of putative chromatin remodelers [3,7].

The DNA-dependent RNA polymerase V (pol V) produces nascent RNA by the transcription of non-coding intergenic regions. The association of pol V to the chromatin requires different components of DDR complex DRD1, DMS3, and RDM1 but the assembly process of these complexes and the mechanism recruiting pol V to chromatin remains unknown [9]. KTF1 binds to the nascent RNA produced by pol V and is tethered to AGO4 by the base pairing between the non-coding RNA transcripts and the siRNAs. The interaction of polV with the WG/GW motif of KTF1 reinforces the association between siRNA and AGO4 forming a functional RdDM complex that directs de novo methylation by activating DNA methyltransferase which in Arabidopsis is called DRM2 [9,10].

In plants, especially in the case of transposons, there is the involvement of nuclear remodeling protein, DDM1. DDM1 is an ATP-driven protein that can assemble nucleosomes, disassemble nucleosomes, move nucleosome and even pick out a part of nucleosomes. Loss of function of DDM1 protein causes major methylation loss in sequence contexts. These DDM1 proteins are required for DNA methylation of transposons [11]. DDM1 is conserved across eukaryotes but its interaction with the RdDM pathway is still unclear and understudy. DDM1 and RdDM are collectively responsible for the methylation of nearly all transposons. DDM1 and RdDM are complementary to each other in methylating transposons [11,12]. For internal methylation of long transposons both DDM1 and CMT2 are required. RdDM is inhibited by heterochromatin so, DDM1 is required for DNA methylation of the heterochromatic transposons.

Maintenance of DNA methylation in plants

The mechanism of maintenance of DNA methylation in plants has been studied extensively. During the semiconservative replication of DNA, the newly synthesized daughter strand lacks methylation. MET1 maintains the CG methylation in plants and the maintenance of CG methylation is similar in plants and animals. In plants CHH and CHG methylation is maintained by CHROMOMETHYLASE 2 & 3 (CMT2 & CMT3), respectively [13,14].

A recent study conducted by Wehdtle *et al.* (2019) proposed a denovo methyltransferase activity for CMT3 at genic loci to establish gene body methylation. In non-flowering plants, a 4th DNA methyltransferase family is found which is lost in angiosperm and represents the orthologous of the mammalian denovo enzyme DNMT3.

In the model plant *Arabidopsis thaliana*, a one-third gene has CG methylation in the coding region and this is maintained by MET1. CG methylation in gene bodies doesn't cause gene silencing as in the case of transposon methylation [5].

CHG methylation in plants is thought to be maintained by the reinforcement loop involving DNA and histone methylation. CMTs interact with H3K9Me2 to catalyze CHG methylation in plants [15]. The loss of CMT3 in mutants has found a dramatic decrease in DNA methylation. Maintenance of CHH methylation is done by DRM proteins but they cannot maintain all CHH methylation [14].

Asymmetrical methylation is maintained by constant de novo methylation by DRM2 and RdMD. This maintenance pathway requires protein with the SRA domain (SUVH9 and SUVH2). These proteins bind CHH and CG methylation sites and are essential for DRM2 mediated de novo maintenance and DNA methylation [13-15].

DNA demethylation in plants

DNA methylation is stable epigenetic marks and heritable both mitotically and meiotically. But these methylation marks are decreased during the developmental process because of the absence or mutation of enzyme maintaining methylation. DNA methylation can occur passively, lacking enzyme maintaining methylation, or actively by removing methylated cytosine from the genome.

Passive DNA methylation

Passive DNA replication occurs during DNA replication. The failure of MET1 to maintain methylation in the newly synthesized daughter strand causes loss of methylation marks in the new daughter strand [16].

In plants, MET1 expression levels are reduced in female gametogenesis. A study in *Arabidopsis thaliana* revealed that the expression of genes MULTICOPY SUPPRESSION OF IRA1 (MSI 1) and RETINOBLASTOMA RELATED 1 (RBR1) have repressed enzyme activity of MET1 [16,17].

Active DNA demethylation

Active DNA demethylation removes methylated cytosine by the activity of DNA glycosylase in combination with the base excision repair (BER) pathway. DNA glycosylase includes DEMETER (DME), REPRESSOR OF SILENCING 1 (ROS1), and others like DML2, DML3 [18,19]. DML proteins are larger DNA glycosylase ranging from 1,100 to over 2,000 residues with the bifunctional activity of DNA glycosylase and apurinic/apyrimidinic (AP) lyase activities [19]. But the process of how these plant 5-meC DNA glycosylases are directed toward the specific genomic sequences is still poorly understood and assumed that it involves the presence of particular chromatin modifications at target loci or the activity of the recruiting factors.

Active demethylation involves the removal of the methylated base by cleavage of the N-glycosidic bond generating of an abasic site. This catalytic activity is performed by DME/ROS1. DEM, ROS1, and DML3 can demethylate 5-meC in any sequence context but they show a preference for CG sequences. ROS1 excises 5-meC more effectively when it is mismatched with A, C, or T then correctly paired [14]. Both phosphodiester linkage (5' and 3') of the abasic site is broken via apyrimidinic (AP) lyase activities, generating a single nucleotide gap in the DNA sequence. This gap possesses an end with 3' -PUA (phosphor- α,β -unsaturated aldehyde) or 3'-P end [27]. These non-canonical terminal ends must be converted into 3'-OH ends to act by DNA polymerase and ligase. The excision of methylated cytosine catalyzed by DEM and ROS1 proceeds with the formation of intermediates Schiff base during the catalytic process [20,21].

The processed gap containing 3'-OH end is acted upon by X-family DNA polymerase- β , But the homology of these enzymes has not been identified in plants. The X-family member DNA polymerase in a plant is DNA polymerase λ (Pol λ) but its role in active DNA demethylation is still undetermined [21,22].

After the addition of the nucleotide in the gap, a complex of DNA ligase (Lig III) and scaffold protein XRCC1 catalyzes DNA ligation in mammals. Three DNA ligases (LIG1, LIG4, and a plant-specific LIG6) are encoded by the *Arabidopsis* genome but lack homologs of Lig III [23]. LIG 1 has been demonstrated to participate in BER ligating gaps in both short and long patch BER [16].

DNA methylation and response to biotic stress in plants

Epigenetic modifications in the plant genome are vital for the plant's survival against biotic stresses like bacteria, fungus, viruses, insects, and to compete with weeds in their natural habitat. Research has revealed epigenetic involvement when plants interact with biotic pathogens and weeds ultimately developing the adaptation mechanism.

Biotic stress in plants induces genome-wide DNA methylation change in response to the infection of pathogen and colonization by symbiotic bacteria. Nodulation by symbiotic bacteria in the *Medicago truncatula* requires demethylase enzyme DEM. Several genes are differently methylated during nodulation in a plant [24,25]. Root infection by cyst nematode in soybean and *Arabidopsis thaliana* have induced widespread DNA hypomethylation [26].

Arabidopsis leaves when infected with the bacterial pathogen *Pseudomonas syringae* pv. tomato str. DC3000 (Pst DC3000) has induced mild widespread differential DNA methylation; this methylated cytosine is mainly found in CG and CHH context [7,26].

In *Arabidopsis*, histone methyl transferase SDG-8 and SDG-25 regulate pp1-, flg22-, and effector-triggered immunity and system acquired resistance when inoculated with *Botrytis cinerea* and *Alternaria brassicicola*. The SDG-8 and SDG-25 are involved in H3K4 and H3K36 methylation during infection [27]. Also, sdg-8 and sdg-25 mutants were impaired in H2B ubiquitination; H2B ubiquitination is involved in the expression of plant immunity genes, accumulation of lipids, biosynthesis of carotenoids, and maintenance of cuticle integrity [27,28].

Viroids are the smallest biological pathogen only with long noncoding RNAs lacking protein coats, causing various diseases in plants. Many researchers have reported that the interaction of viroids with plants has induced genome-wide epigenetic modifications. An increased rRNA expression rate is reported in cucumber and a close relative of tobacco when infected by Hop stunt viroid (HSVd) that results from reactivation of the rRNA by DNA demethylation in the promoter region of some ribosomal genes in cucumber leaves and pollen grains [29,30].

A genome-wide study of *A. thaliana* suggests that biotic stress in plants is a major factor shaping epigenome [26]. Mutational changes in the regulator of DNA methylation and demethylation can alter the plant susceptibility to pathogen infestation. Mutation of the pol V enzyme involved in the RdDM pathway decrease resistance to necrotic fungal infestation (*Botrytis cinerea* and *Plectosphaerella cucumerina*) [31].

DNA methylation and response to abiotic stress

Researchers have revealed the importance of DNA methylation and demethylation to cope with the abiotic stress in plants. These abiotic stresses include heat, cold, drought, flood, snow, nutrient deficiencies, pesticides, and climate change. These stresses induce DNA methylation and demethylation which is genome-wide or locus-specific [32]. Vernalization memory of the cold treatment requiring crop is one of the best examples of DNA methylation responding to abiotic stress. Vernalization causes the suppression of the transcription of flowering locus C (FLC) via recruitment of H3K27me3 in a polycomb response element (PRE) and H3K27me3 over the entire 7-kb FLC locus when plants are at warm temperatures [28].

Cold treatment of tomato fruits disturbs the DNA methylase DML2. Downregulation of DML2 causes hypermethylation and silencing of the gene responsible for the biosynthesis of flavor volatiles in tomato, explaining the flavor loss of tomato during cold storage [33].

Global warming has increased the temperature of the globe producing stress in plants reducing crop yields. Research has revealed the epigenetic mechanism underlying the heat response in plants. Temperature stress in plants leads to the H3K4 methylation; this H3K4 methylation is involved in the continuous expression of the high-temperature responsive gene [34] when the plant is exposed to prolonged heat stress, serving as a memory to cope with heat stress in recurring situations [28,35]

Nutrient deficiencies and toxicities produce stress in plants reducing plant yield. Abnormal supply of Nitrogen, Phosphorus, and Iron in soil have induced H3K27me3, H4R3me2, H3K4me3 methylation, and H3K9ac, H3K14ac acetylation [36]. In rice, lack of inorganic phosphate generates more than 100 differently methylated regions in the genome which are mainly CHH hypermethylation. The promoter of some endogenous genes is enriched with methylation marks as a result of abiotic stress. Maize also shows methylation patterns similar to rice in response to abiotic stresses [37]. DNA methylation was found to regulate the salinity and heavy metal stress in barley and wheat. The methylation level at the promoter region of stress-responsive gene TaFLS1 was lower leading to higher gene expression in stress-tolerant wheat and barley [35].

In *A. thaliana*, the induced change in DNA methylation by high salinity stressed is partly transmitted to the progeny. If these progenies are not given stress, then these methylation marks are gradually reset in progeny. A requirement of continuous stress to persist stress-induced epigenetic memory was observed in the SDS gene which encodes SUPPRESSOR OF DRM1, DRM2, CMT3, and is silenced by DNA methylation in the promoter region. This SDS gene is activated after heat treatment and repeated heat treatments are required for SDS re-silencing [38].

Conclusions

Recent advancements in genomic study and sequencing technologies have provided a platform to study the DNA methylation pattern and its role in plants. The RdDM pathway of de novo methylation which involves the use of non-coding RNAs for methylation in plants, the pathway, and proteins involved in active DNA demethylation for activation of the different genes in the different developmental process has been developed recently. Scientists explored the DNA methylation patterns and the response of plants towards various biotic and abiotic stresses and have used this knowledge to manipulate plant traits for human kinds. Apart, recent advancement and discoveries in this field have also highlighted new unsolved questions in front of all scientists. Gene inactivation is done by promotor methylation which inhibits the binding of transcription activator but in some cases, promotor methylation activates the gene, how this activation occurs is still unknown. In de novo methylation by the RdDM pathway, small RNAs determine target specificity for methylation, these small RNAs also mediate the targeting of ROS1 which is involved in demethylation at several ROS1 dependent genomic regions. How these small RNAs guides both methylation and demethylation patterns are still unclear and need further study. Different complexes of DDR (DRD1, DMS3, and RDM1) are required for the association of pol V to the chromatin but the assembly process of these complexes and the mechanism recruiting pol V to chromatin remains unknown. DDM1 is involved in CHH methylation in plant and is conserved across eukaryotes but its interactions with the RdDM pathway is still unclear.

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