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Review

An update on histone lysine methylation in plants

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Abstract

Histone methylation plays crucial roles in epigenetic regulation. The SET domain proteins are now recognized as generally having methyltransferase activity targeted to specific lysine residues of histones. The enzymes and their specific histone lysine methylation have enormous impacts on the regulation of chromatin structure and function. In this review, we discuss recent advances made on histone lysine methylations and their diverse functions in plant growth and development.

Keywords: Chromatin modification; Histone; Lysine methylation; SET domain protein; Plant

1. Introduction

Recently, study on chromatin modifications has become a hot research topic. This is primarily because the high-order chromatin structure has an important role in controlling gene expression and in maintenance of genome integrity. Chromatin is a complex structure built from repeating units, the nucleosomes. Histones, as the structural core of the nucleosome, are subjected to multiple types of covalent modifications, such as acetylation, methylation, phosphorylation, and ubiquitination. These histone modifications represent additional epigenetic information on chromatin and have been proposed to form a "histone code" that can be recognized by specific regulatory proteins or protein complexes which participate in the regulation of the transcriptional activity of an embedded gene.

Histone lysine methylation can occur on lysine residues 4, 9, 27, 36, and 79 of histone H3, and on lysine 20 of histone H4. Furthermore, lysine residues can be mono-, di-, or trimethylated, which add another layer of complexity of histone code information. In general, methylation on H3K4, K36, and K79 correlates with active transcription, whereas methylation on H3K9, K27, and H4K20 associates with heterochromatinization and gene silencing.

Many proteins responsible for histone methylation of specific lysine residues have been characterized. Apart from the DOT1 and DOT1L, which methylate H3K79 in the globular region, all histone methyltransferases (HMTases) contain a conserved SET domain of about 130 amino acids in length. The name SET comes from the first letter of three *Drosophila* genes involved in epigenetic processes: *Su(var)*, *E(z)*, and *Trithorax*. A number of studies indicate that SET domain proteins have HMTase activities and are involved in the regulation of gene expression, positively or negatively by catalyzing distinct histone substrates.

Here, we summarize the recent advances in the study of histone lysine methylation and SET domain proteins in plants. Specific examples are discussed, in which the influence on plant development is understood.

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2. Plant SET domain proteins

Plants contain a great number of genes encoding SET domain proteins. According to the annotation from the databases Pfam and ChromDB (http://www.chromdb.org/), at least 47, 35, and 37 SET domain proteins are present in *Arabidopsis*, maize and rice, respectively. Upon sequence analysis, Ng et al. [1] grouped the plant SET domain proteins into I–VII classes: class I—E(z) class; class II—ASH1 class; class III—Trithorax class; class IV—proteins with a SET and a PHD domain; class V—Su(var) class; class VI—proteins with an interrupted SET domain; class VII—non-histone methyltransferases and related proteins.

Considering that little is known about the function of proteins in the class IV and class VI, and that the class VII proteins are likely non-histone methyltransferases, here we focus on class I, class II, class III, and class V proteins.

2.1. Class I—E(z) class (H3K27 methylation)

E(z) is a *Drosophila* PcG (Polycomb Group) protein and functions as a HMTase to methylate K27 of histone H3 (H3K27). The methylated H3K27 could further bind with the chromodomain subunit of the polycomb repressive complex 1 (PRC1), resulting in stable silencing of transcription [2].

The *Arabidopsis* CLF (Curly Leaf, also named SDG1), SWN (Swinger, also named SDG10), and MEA (Medea, also named SDG5) proteins have been well characterized in plants (Table 1). *CLF* is the first SET-domain-encoding gene characterized in plant. CLF controls leaf and flower morphology as well as flowering time through repression of the floral homeotic genes *AG* (*Agamous*) and class I *KNOX* gene *STM* (*Shootmeristemless*) by H3K27 trimethylation [3]. SWN, as the closest homolog of CLF, acts redundantly with CLF in mediating H3K27 trimethylation at both *AG* and *STM* loci [3]. *MEA* is the first imprinting gene found in the *Arabidopsis* endosperm. The *mea* mutant causes endosperm overproliferation, arrests embryo development and seed abortion when maternally inherited. Imprinting of the *MEA* in the female gametophyte is controlled by antagonism between DNA methyltransferase MET1 and DNA glycosylase DME (Demeter) [4]. DME establishes *MEA* imprinting by removing 5-methylcytosine to activate the maternal allele. MEA as well as other PcG proteins from the maternal genome might control paternal *MEA* silencing [5]. Furthermore, MEA could bind with other proteins such as FIS2 (Fertilization Independent Seed2), FIE (Fertilization Independent Endosperm), and MSI1 (Multicopy Suppressor of IRA1) forming a polycomb repressive complex similar to animal PRC2 in seed development [6,7]. A target gene of the MEA/FIE/FIS2 complex, which encodes type I MADS domain protein Pheres1, has been identified [8].

The rice homolog of CLF, OsSET1/OsiEZ1 has also been characterized [29,30]. Overexpression of OsSET1 affects shoot development in transgenic *Arabidopsis*.

2.2. Class II—ASH1 class (H3K36 methylation)

The typical member of this class is the yeast SET2, which is a H3K36-specific HMTase and associates with

Table 1
Sites and functions of histone lysine methyltransferases in plants.

Site	HMTases	Functions	References
H3			
K4	ATX-1(*At*)	Transcriptional activation	[9–13]
	ATX2(*At*)	Transcriptional activation	[13]
	SDG4(*At*)	Floral organ development	[14,15]
K9	KYP(*At*)	DNA methylation	[16–20]
	SUVH6(*At*)	DNA methylation at *PAI1–PAI4* locus	[19]
	SUVH5(*At*)	DNA methylation	[20]
	SUVH2(*At*)	Pericentric heterochromatin	[21]
	SUVR4(*At*)	Putative role in controlling rRNA expression	[22]
	SDG714(*Os*)	DNA methylation, transposition inhibition	[23,24]
	NtSET1(*Nt*)	Pericentric heterochromatin	[25–28]
K27	MEA(*At*)	Gene imprinting in endosperm	[4–8]
	CLF(*At*)	Repression of the floral homeotic genes	[3,11]
	SWN(*At*)	Repression of the floral homeotic genes	[3]
	SUVH2(*At*)[a)]	Pericentric heterochromatin	[21]
	OsSET1(*Os*)	Unclear function in plant development	[29,30]
K36	SDG4(*At*)	Floral organ development	[14,15]
	SDG8(*At*)	Transcriptional regulation, flower time control	[31–33]
	SDG26(*At*)	Transcriptional regulation, flower time control	[32]
H4			
K20	SUVH2(*At*)[b)]	Pericentric heterochromatin	[21]

Species abbreviations: At, Arabidopsis thaliana; Nt, Nicotiana tabacum; Os, Oryza sativa

a) and b) need to be further demonstrated.

the phosphorylated C-terminal domain (CTD) of RNA polymerase II (RNAPII), implying that this enzyme might play an important role in the transcription elongation process [34].

The identified *Arabidopsis* members grouped in this family include SDG8/EFS, SDG26 and SDG4/ASHR3 (Table 1). Shen's lab first demonstrated the role of H3K36 methylation mediated by SDG8 in eukaryote development [31]. SDG8 is a homolog of the yeast SET2, and is specifically required for di- and trimethylation on H3K36 in *Arabidopsis* [31,32]. The loss-of-function mutant *sdg8* results in reduced H3K36 methylation, particularly in the promoter region and the first intron of *FLC* chromatin. The *sdg8* mutants display reduced *FLC* expression and flower early, indicating that H3K36 methylation mediated by SDG8 is an important epigenetic memory code required for *FLC* expression in preventing early flowering. Besides early flowering, the *sdg8* mutants show an additional pleiotropic phenotype such as the reduced plant size and fertility [31–33], suggesting that the contribution of SDG8 is not limited to flowering time control. Nevertheless, in contrast to the early flowering phenotype of the *sdg8* mutants, the *sdg26* mutants show a late-flowering phenotype due to the up-regulated *FLC* expression [32]. Microarray analysis indicates further that SDG26 is primarily involved in maintaining the repressed state of genes, whereas SDG8 is primarily involved in maintaining the activated state of genes [32]. Another characterized *Arabidopsis* member of this class, SDG4, is expressed specifically in floral organs and is involved in H3K4 and H3K36 methylations [14]. Furthermore, SDG4 was shown to bind the transcription factor AMs (aborted microspores) and thus proposed to be targeted to chromatin and to regulate gene expression by association with AMs in flower development [15].

2.3. Class III—Trithorax class (H3K4 methylation)

In contrast to PcG proteins, TrxG (trithorax group) proteins are identified genetically as a positive regulator of homeotic genes. *Drosophila* Trx-G protein TRX (trithorax), possessing H3K4-specific HMTase activity, can regulate homeotic gene expression via TAC1 (trithorax acetyltransferase complex1) protein complex [35]. Similarly, the yeast homolog SET1 functions in transcriptional activation via protein complex COMPASS (complex proteins associated with SET1) [36].

The *Arabidopsis* proteins involved in this class are also named ATX (*Arabidopsis* trithorax) and ATXR (*Arabidopsis* trithorax related). The well-characterized one is ATX1/SDG27, bearing H3K4 HMTase activity [9]. Nevertheless, ATX1 seems not involved in the overall methylation of H3K4 but in the modification of only a fraction of *Arabidopsis* histones embedded in the flower homeotic genes [9,10]. Flower homeotic gene *AG* is demonstrated to be controlled by ATX1 and CLF together, indicating that the H3K4 methylation by ATX1 and the H3K27 methylation by CLF establish 'bivalent chromatin marks' at the silent *AG* locus to regulate *AG* expression in antagonistic manner [11]. Recently, a specific trimethylation function of ATX1 on H3K4 in controlling *FLC* activation has been reported [12]. Although structurally similar to *ATX1*, *ATX2* encodes protein with divergent functions: ATX2 dimethylates K4 of histone H3 and influences the expression of largely nonoverlapping gene sets of ATX1 [13].

2.4. Class V—Su(var) class (H3K9 methylation)

The Su(var) class comprises the largest number of plant SET domain proteins (http://www.chromdb.org), at least 15 SET domain proteins are present in *Arabidopsis*. The functionally characterized *Arabidopsis* members in this class include KYP (kryptonite)/SUVH4/SDG33, SUVH5/SDG9, SUVH6/SDG23, SUVH2/SDG3, and SUVR4/SDG31 (Table 1). Genetic analyses revealed that KYP is involved in the transcriptional silencing of euchromatic loci, including the floral homeotic gene *Superman* and the tryptophan biosynthetic genes *PAI2* and *PAI3* [16,17], as well as in H3K9 methylation within the heterochromatic chromocenters [18]. In contrast, mutations in KYP do not affect H3K9 methylation and DNA methylation at the *PAI1–PAI4* transcribed inverted repeat. However, both epigenetic modifications are reduced at this locus in a *kyp suvh6* double mutant [19], suggesting that H3K9 methylation and DNA methylation are maintained in the transcribed inverted repeat by combined action of KYP and SUVH6 HMTases. The SUVH5 protein also has HMTase activity *in vitro* and contributes to the maintenance of H3K9 methylation and CMT3-mediated non-CG methylation together with KYP and SUVH6 *in vivo* [20]. However, the relative contributions of KYP, SUVH5, and SUVH6 to non-CG methylation are locus specific. SUVH5 and KYP together control the transposon sequences with only a minor contribution from SUVH6. Another characterized *Arabidopsis* member of this class is SUVH2 [21]. In *SUVH2* null plants, mono- and di-methyl H3K9, mono- and di-methyl H3K27, and mono-methyl H4K20, the histone methylation marks of *Arabidopsis* heterochromatin are all significantly reduced, suggesting that SUVH2 has a central function in heterochromatic gene silencing in *Arabidopsis*. Recently, SUVR4, which also belongs to the Su(var) class but differs from the SUVH proteins in their domain structures, has been demonstrated to monomethylate H3K9 [22]. GFP-fused SUVR4 protein is preferably localized in the nucleolus, suggesting a possible role of SUVR4 in the regulation of rRNA expression.

Cao's lab [23] and us [24] reported that the rice *SDG714* encodes a histone H3K9-specific HMTase. The loss of function of *SDG714* resulted in decreased levels of H3K9 methylation and DNA methylation at the retrotransposon *Tos17* locus, causing the activation of *Tos17* transposition [23]. The rice SDG704 and SDG706 did not show HTMase activity in our *in vitro* assay conditions. All the characterized three rice proteins are localized in the nucleus, with

SDG704 and SDG714 being stably associated with mitotic chromosomes [24].

The tobacco SET domain protein NtSET1, which was previously found to associate with chromatin belongs also to the Su(var) class [25]. In Shen's lab, we reported that NtSET1 also methylates H3K9, and that the ectopic expression of NtSET1 induces chromosome-segregation defects in tobacco BY2 cells [26] and inhibits plant growth [25,27]. In addition, NtSET1 can bind LHP1, the *Arabidopsis* homolog of the animal heterochromatin protein1 (HP1), in both yeast two-hybrid and pull-down assays [26]. More recently, the association of NtSET1 and LHP1 with heterochromatin DNA sequences has been demonstrated by chromatin immunoprecipitation (ChIP) analysis [28].

3. The diverse functions of histone lysine methylation

3.1. Histone lysine methylation and transcriptional activation

In general, both H3K4 and H3K36 methylations are associated with transcriptional active euchromatin, but they exert at different steps during transcription process. Both di- and tri-methyl H3K4s are distributed within transcription units, with tri-methyl H3K4 being enriched in fully activated promoters, which have been demonstrated in yeast and animals [37]. Similarly, the distribution of di- and tri-methyl H3K36s is also detected in active genes, whereas H3K36 methylation is initiated after transcriptional initiation and occurs at an approximately fixed distance from the initiation site of transcription [38].

The model in yeast might explain the mechanisms of H3K4 and H3K36 methylations in transcriptional activation [39]. Firstly, the ubiquitination of H2BK123 facilitates the recruitment of the H3K4 HMTase SET1, leading to H3K4 methylation and the formation of Paf1 elongation complex, coinciding with phosphorylation of serine 5 of the carboxy-terminal domain (CTD) of RNA polymerase II. H3K4 methylation subsequently recruits SAGA (Spt-Ada-Gcn5-acetyltransferase) complex, which contains the Ubp8, a deubiquitination factor. The subsequent deubiquitination by Ubp8 allows the recruitment of SET2, which methylates H3K36, coinciding with CTD serine 2 phosphorylation. Thus, H3K4 methylation seems to be involved in earlier events of transcription, whereas H3K36 methylation is more closely associated with transcription elongation. However, this yeast model of different types of ordered histone modifications associated with different steps of transcription initiation and elongation remains to be verified in plants.

The analysis in *Arabidopsis* on the *phas* (*phaseolin*) promoter shows that methyl H3K4, particularly tri-methyl H3K4 at the *phas* promoter is significantly increased when the *phas* promoter is actively transcribed [40]. Di-methyl and tri-methyl H3K36s caused by *Arabidopsis* SDG8 are enriched in the *FLC* promoter and the first intron, which is required for the expression of *FLC* [31,32]. Although the association of SDG8 with RNA polymerase II is not yet demonstrated, SDG8 contains two motifs that are conserved within the RPB1 subunits of RNA polymerase II, suggesting its associating role with the transcription machinery.

3.2. Histone lysine methylation and transcriptional repression

In mammalian cells, mono- and di-methyl H3K9s are preferentially localized to euchromatin and are crucial epigenetic marks for transcriptional silencing. G9a and G9a-related HMTase GLP, belonging to the Su(var) class in mammals, are shown to be responsible for H3K9 mono- and dimethylation and are involved in the repression of euchromatic genes [41]. In addition, evidence comes from the finding that the tumor suppressor retinoblastoma (Rb) protein can recruit some chromatin modification complexes such as Suv39h1/HP1 to the cell cycle gene *cyclin E* to repress gene transcription [42]. However, unlike mammalian, tri-methyl H3K9 instead of di-methyl H3K9 is found at *Arabidopsis* euchromatin regions. Although similar HMTases like G9a have not been identified in plants, some plant HMTases are found to associate with euchromatin gene silencing. For example, H3K9 HMTase KYP is involved in the transcriptional silencing of the floral homeotic gene *Superman* and the tryptophan biosynthetic genes *PAI2* and *PAI3* [16,17]. Our results showed that NtSET1-GFP protein is broadly distributed in tobacco BY2 cells, covering in part both heterochromatic foci and euchromatin regions [26], suggesting a possible role for NtSET1 in euchromatin gene silencing.

In addition to H3K9 methylation, H3K27 methylation serves as an epigenetic mark for PcG-mediated gene silencing. In *Arabidopsis*, vernalization-induced H3K27 dimethylation causes the down-regulation of *FLC* transcription [43]. Moreover, H3K27 trimethylation is required for the maintenance of the repression state of some crucial genes involved in *Arabidopsis* development, such as *AG*, *STM*, and *Pheres1* [3,8]. Genome profiling revealed that H3K27 trimethylation is associated with a great number of euchromatic genes [44], which are specifically repressed spatially and temporally due to the H3K27 trimethylation. LHP1, likely through its chromodomain can bind with tri-methyl H3K27 and may play an analogous role of animal PRC1 in euchromatin gene silencing [45,46].

3.3. Histone lysine methylation and DNA methylation

Besides histone methylation, DNA cytosine methylation provides another heritable epigenetic mark, and is involved in genomic imprinting and regulation of gene expression. Cytosine methylation is mostly restricted to symmetrical CG di-nucleotide context in vertebrates. In contrast, DNA methylation in plant occurs at CG, CNG (where N is any nucleotide) and CHH (where H is A, C, or T) asymmetric sequences, each of which has different genetic requirements for the maintenance of its methylation [47].

Arabidopsis contains three types of DNA methyltransferases: MET1 (methyltransferase1) functions in CG methylation; CMT3 (chromomethylase3) controls the major plant-specific CNG methylation; DRM1 (domains rearranged methyltransferase1) and DRM2 function to maintain asymmetric CHH methylation and to control *de novo* methylation [47].

Previous studies showed that H3K9 methylation is required for DNA methylation in several species. In *Neurospora*, mutations in *DIM-5,* which is shown to encode an H3K9-specific HMTase, result in abnormal growth and complete loss of DNA methylation [48]. The same causal relationship is also reported in plant. Loss-of-function alleles of *kyp* result in reduced DNA methylation specifically at non-CG sites [16], as in *cmt3* mutants, suggesting that CMT3 activity occurs downstream of H3K9 methylation. In addition, it has been shown that simultaneous methylation of H3K9 and H3K27 is recognized by CMT3 [49], implying that methylation of H3K9 and H3K27 forms a combinatorial histone mark required for non-CG methylation.

However, some other results seem to contradict previous scenario in *Neurospora* and *Arabidopsis*. It was shown that the methylation level of H3K9 in heterochromatin is drastically reduced after removal of CG methylation in *Arabidopsis met1* mutant lacking the DNA methyltransferase MET1 [50,51]. While H3K4 dimethylation and H3K27 trimethylation, both of which are euchromatic marks in *Arabidopsis*, are enriched upon loss of CG methylation [52]. However, mono- and dimethylation of H3K27, which are the heterochromatin marks in *Arabidopsis*, are not influenced by DNA CG methylation [52]. Does it mean that MET1-mediated CG methylation is required for the retention of H3K9 methylation? Our recent results demonstrate that the tobacco H3K9 HMTase NtSET1 can bind the DNA methylcytosine-binding protein VIM1 (variant in methylation1) [28]. Moreover, the distinct SRA domains of KYP and other plant Su(var) class proteins were shown to bind directly with methylated DNA [53], suggesting that DNA methylation is required for the recruitment of Su(var) class proteins such as KYP.

Taken together, we propose a model to summarize the relationship between histone methylation and DNA methylation in *Arabidopsis* (Fig. 1). CG methylation by MET1 recruits directly or indirectly H3K9 histone HMTases to stimulate H3K9 methylation. H3K9 methylation, together with H3K27 mono- and dimethylation, forms a combinatorial histone mark required for the recognition of CMT3, which in turn induces non-CG methylation. In addition, since SRA domain binds non-CG methylation sites in addition to CG methylation sites [53], there is likely a self-reinforcing loop between histone methylation and DNA non-CG methylation [28].

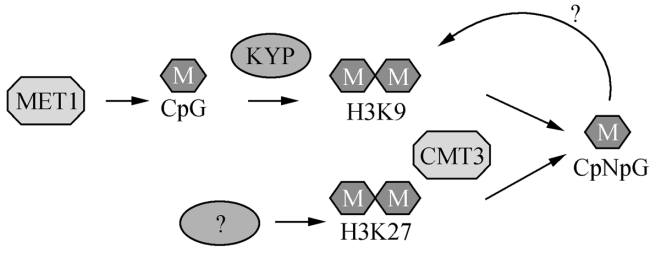

Fig. 1. Summary of the relationship between histone methylation and DNA methylation in *Arabidopsis*.

3.4. Histone lysine methylation and heterochromatin

Heterochromatin is characterized by the histone H3K9 methylation and DNA cytosine methylation. An intrinsic relationship between H3K9 methylation and heterochromatin is first established by the discovery of the mammalian Su(var) class protein Suv39h1. The Suv39h1 methylates specifically histone H3K9, which creates a binding site for the HP1 and results in heterochromatin formation [54]. Our studies discovered a link between tobacco H3K9 HMTase NtSET1 and LHP1 [26,28]: NtSET1 binds LHP1; NtSET1 and LHP1 bind centromeric/pericentromeric sequences including centromeric repeats and the retrotransposon *Ta3* in *Arabidopsis*; and NtSET1 and LHP1 bind telomere/subtelomere-like sequences in tobacco. Similarly, the mammalian Suv39h1 and HP1 are demonstrated to be involved in heterochromatin formation of centromere and telomere [55]. We could not yet conclude that NtSET1 and LHP1 target telomere because telomere/subtelomere-like sequences are found, in addition to telomeric regions, in centromeric regions of some *Solanum* species [56]. Nevertheless, NtSET1 and LHP1 were shown to be localized in heterochromatin regions, suggesting a similar mechanism in tobacco as in animals. The role of LHP1 in heterochromatin is still controversial. The *Arabidopsis lhp1* mutant did not show significant changes in chromocenter organization as viewed by DAPI staining and FISH with CEN180 [57], nor in expression of genes within centromeric regions [58]. It is possible that LHP1 functions in heterochromatin redundantly with other proteins.

In addition to H3K9 methylation, mono- and di-methyl H3K27 and mono-methyl H4K20 are also found to associate with heterochromatin in *Arabidopsis*. In *SUVH2* null *Arabidopsis*, all these histone methylation marks are significantly reduced [21]. Consistently, overexpression of *SUVH2* causes obvious heterochromatization [21]. However, how the methylation of H3K27 and H4K20 are involved in heterochromatin is still unclear in plant. The analysis on mammalian shows that the H4K20 HMTase Suv4-20h binds HP1 *in vitro* [59], suggesting that Suv4-20h is likely recruited by HP1 into heterochromatin regions to mediate H4K20 methylation.

4. Perspectives

Histone lysine methylation plays a crucial role in plant epigenetic regulation, and has important functions in many biological processes including cell proliferation and differ-

entiation, transcriptional regulation, and heterochromatin formation. Recently, the field of histone methylation has flourished with the identification of numerous HMTases. Compared with the research in yeasts and animals, more questions need to be addressed in plants. Plants contain a great number of genes encoding SET domain HMTases, among which only a few of them have been characterized. The numerous HMTases surely have divergent functions in biological processes. So the challenge is to uncover their functions in plant development and to define the molecular mechanism that links histone lysine methylation with specific biological processes. Furthermore, the findings of histone demethylases extend the complexity of dynamics on histone lysine methylation. Finally, the histone lysine methylation cross-talks with other types of histone modifications. These outline issues need to be addressed in the future studies.

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