# Histone Methylation in Higher Plants

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## Key Words

histone methylation, histone demethylation, protein arginine methyltransferase (PRMT), SET domain protein, JmjC domain–containing protein, histone demethylase

## Abstract

Histone methylation plays a fundamental role in regulating diverse developmental processes and is also involved in silencing repetitive sequences in order to maintain genome stability. The methylation marks are written on lysine or arginine by distinct enzymes, namely, histone lysine methyltransferases (HKMTs) or protein arginine methyltransferases (PRMTs). Once established, the methylation marks are specifically recognized by the proteins that act as readers and are interpreted into specific biological outcomes. Histone methylation status is dynamic; methylation marks can be removed by eraser enzymes, the histone demethylases (HDMs). The proteins responsible for writing, reading, and erasing the methylation marks are known mostly in animals. During the past several years, a growing body of literature has demonstrated the impact of histone methylation on genome management, transcriptional regulation, and development in plants. The aim of this review is to summarize the biochemical, genetic, and molecular action of histone methylation in two plants, the dicot *Arabidopsis* and the monocot rice.

## Contents



**Histone code hypothesis:** different histone modifications function on the same or adjacent histone tails in a combinatorial or sequential manner; these modifications are read by other proteins, thereby producing specific downstream functions

**Vernalization:** the process in which seeds or seedlings are exposed to prolonged cold or winter conditions to promote the floral transition

## INTRODUCTION

In eukaryotes, genomic DNA is tightly compacted into a complex structure known as chromatin; chromatin structure is a key regulator that influences the accessibility of factors and cofactors for all DNA-templated processes. The fundamental unit of chromatin is the nucleosome, which is composed of ~146 base pairs of DNA wrapped on a histone octamer (containing two copies of each of the four histone proteins H2A, H2B, H3, and H4). The structure and function of chromatin are regulated by multiple epigenetic mechanisms, including histone modification, DNA methylation, ATP-dependent chromatin remodeling, placement of histone variants, and regulation by noncoding RNA. Posttranslational covalent histone modifications, together with small interfering RNA (siRNA) and DNA methylation, are implicated in modulating chromatin structure and gene activity. The amino-terminal tails of the core histones are subjected to various posttranslational modifications, including acetylation, methylation, ubiquitination, phosphorylation, glycosylation, ADP-ribosylation, and sumoylation. The histone code hypothesis predicts that these covalent modifications might provide specificity for effector proteins that bind the modification marks and interpret the code into functional outcomes (51). Although most of the histone modifications are conserved across different kingdoms, the establishment and maintenance of these modifications in plants are related but not identical to fungi and animals (8, 64, 86, 101, 154). For example, the double fertilization of flowering plants, in which the egg cell is fertilized by one sperm to form the embryo while a second sperm fertilizes the two polar nuclei to form the endosperm, allows for unique mechanisms of epigenetic inheritance. Also, the floral transition makes permanent changes in shoot apical meristem identity and provides an excellent example of an epigenetically regulated event that is determined by developmental and environmental cues (e.g., vernalization). Here, we review the current knowledge of the biochemical, genetic, and developmental properties of histone methylation and demethylation in *Arabidopsis* and rice, with emphasis on (*a*) the enzymes responsible for adding or removing the covalent methylation marks; (*b*) the functions of these writers, readers, and erasers of histone methylation (**Figure 1**) in the regulation of gene activity and plant development; and (*c*) the role of these epigenetic modifications in establishing heterochromatin environments and the effect of environmental signals on histone methylation and floral transition in *Arabidopsis*, using *FLOWERING LOCUS C* (*FLC*), which encodes a central flowering repressor, as an example.

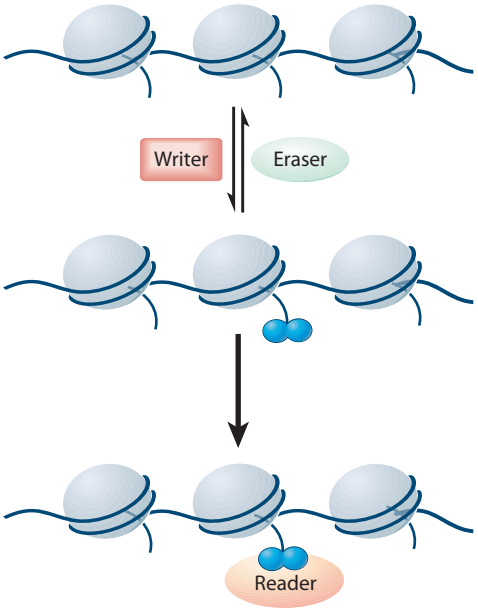


**Figure 1**

Schematic representation of the processes of writing, reading, and erasing the histone posttranslational modifications. Writer enzymes add marks to histones and these marks are removed by eraser enzymes. To produce a biological readout, reader proteins specifically bind to the histone modification. The round shape in grey represents histones. The peanutlike shaped mark in blue represents the specific histone modifications.

## WRITERS OF HISTONE METHYLATION

Histone methylation plays an essential role in diverse biological processes ranging from transcriptional regulation to heterochromatin formation. As one of the most complex modifications, it not only occurs at different residues (lysine and arginine) and distinct sites but also differs in the number of methyl groups added. Unbiased mass spectrometry in combination with high-performance liquid chromatography (HPLC) separation has been used to identify the histone modification profile in *Arabidopsis*, revealing both conserved and nonconserved modifications compared to animals (57, 147). In *Arabidopsis*, histone lysine methylation occurs mainly at Lys4 (K4), Lys9 (K9), Lys27 (K27), and Lys36 (K36) of histone H3. These modifications are written by different histone lysine methyltransferases (HKMTs) (**Table 1**). In contrast to mammals and yeast, in which Lys20 (K20) of histone H4 (H4K20) is methylated, H4K20 is acetylated in *Arabidopsis*, though mono-methylated H4K20 (H4K20me1) has been reported to be detected by immunostaining (94). Another difference is the lack of an *Arabidopsis* homolog of DOT1, an H3 lysine 79 (H3K79) methyltransferase required for telomeric silencing in mammals and yeast; nor is any H3K79 methylation detected (147). In addition, *Arabidopsis* and rice were shown to have much higher levels of H3K4 di-methylation (H3K4me2) than mouse and human, and *Arabidopsis* has much lower H3K9me2 and H3K9me3 levels (42, 47). These results indicate that global histone modification levels and patterns in *Arabidopsis* and rice are quite different from those in mammals, possibly reflecting differences in genome composition.

## Lysine Methylation by HKMTs

Lysine methylation of histones is an important and complex epigenetic mark that decorates both transcriptionally silenced and active chromatin domains, depending on which lysine residues are methylated and the degree of methylation. Lysine methylation does not affect the net charge of the modified residues, but it elevates the hydrophobicity and may alter intra- or intermolecular interactions or create new binding surfaces for reader proteins that bind preferentially to the methylated domain. Generally, histone H3K9 and H3K27 methylation is associated with silenced regions, whereas H3K4 and H3K36 methylation is associated with active genes (10). SET domain proteins are putative candidates for the writers of lysine methylation (**Figure 2*a***). The *Arabidopsis* and rice genomes encode 41 and 37 SET domain proteins, respectively (For more details, please see **http://www.chromdb.org**) (38). Based on the homology of SET domains with proteins in animals and yeast, and the

**Writer:** an enzyme that is responsible for adding a posttranslational modification(s) into a given protein (e.g., HKMT)

**Reader:** a protein or protein complex that recognizes and binds specifically to a particular posttranslationally modified substrate

**Eraser:** an enzyme that removes a posttranslational modification(s) from a given protein (e.g., HDM)

**HKMT:** histone lysine methyltransferase

**SET domain:** a conserved motif containing approximately 130 amino acids which was originally identified in three Drosophila proteins: SU(VAR)3–9, Enhancer of zeste [E(Z)], and Trithorax (Trx)

**Table 1 Known writers, readers, and erasers of histone methylation in *Arabidopsis* and rice**

| Sites | Writer | Reader/Effector | Eraser |
|---|---|---|---|
| H3K4 | ATX1(2, 102, 107, 108)<br>ATX2 (108)<br>SDG4 (17) | ORC1/PHD (26)<br>AtING/PHD (70)<br>AL/PHD (70)<br>WDR5a/WD40 (52) | LDL1 (120)<br>JMJ14 (80)<br>Possibly FLD (54, 78)<br>Possibly LDL2 (54)<br>MEE27/JMJ15 (**Figure 5**) |
| H3K9 | KYP/SUVH4 (47, 48, 85)<br>SUVH5 (33)<br>SUVH6 (32, 33, 47)<br>SUVR4 (132)<br>OsSDG714 (29–30) | ND | OsJMJ706 (123)<br>IBM1/JMJ25 (109)<br>Possibly REF6 (145) |
| H3K27 | ATXR5 (49)<br>ATXR6 (49)<br>CLF (39, 53, 112, 113)<br>MEA (41)<br>SWN (20, 138) | LHP1/chromodomain (34, 136, 150) | ND |
| H3K36 | SDG4 (17)<br>SDG8 (31, 143, 153)<br>SDG26 (143) | ND | ND |
| H3R17 | AtPRMT4a,4b (96) | ND | ND |
| H4R3 | AtPRMT1a,1b (144)<br>AtPRMT5/SKB1 (100, 111, 139)<br>AtPRMT10 (95) | ND | ND |

Abbreviation: ND, not determined.

characteristics of the SET domains, cysteine-rich regions, and additional conserved domains, SET domain proteins in plants are classified into four categories, namely, (*a*) SU(VAR)3–9 groups [including SU(VAR)3–9 homologs (SUVH) and SU(VAR)3–9 related proteins (SUVR)], (*b*) E(Z) (enhancer of zeste) homologs, (*c*) TRX (trithorax) groups (TRX homologs and TRX-related proteins), and (*d*) ASH1 (absent, small, or homeotic discs 1) groups [ASH1 homologs (ASHH) and ASH1-related proteins (ASHR)] (7, 122, 152). Although the enzymatic activity and specificity of these plant SET proteins are not known in every case, genetic data suggest that they may act on the same lysine residues or related pathways to the homologous proteins or protein complexes in animals or yeast. The known functions from each class will be discussed in detail below.

**E(Z):** enhancer of zeste

**Chromocenters:** highly condensed constitutive heterochromatin regions mainly composed of pericentromeric repeats, transposable elements, and ribosomal DNA with high levels of cytosine methylation and enriched 24nt siRNAs

## H3K9 Methylation Is a Silencing Mark Linked to DNA Methylation

The histone H3K9 methylation in animals occurs at mono-(H3K9me1), di-(H3K9me2), and tri-(H3K9me3) levels and each level of methylation produces different outcomes (86). In *Arabidopsis*, histone H3K9 methylation exists predominately as H3K9me1 and H3K9me2, while a little H3K9me3 can be detected (57). Immunostaining of nuclei and chromatin immunoprecipitation (ChIP) assays found that H3K9me1 and H3K9me2 is enriched in chromocenters, indicating a conservation of histone marks in silenced chromatin among different species (36, 47, 87). Consistent with the primary function of H3K9me2 in repressing transposon activities, genomewide ChIP assays coupled with high-resolution microarray analysis (ChIP-chip) revealed that H3K9me2 is enriched in transposons and repeated sequences

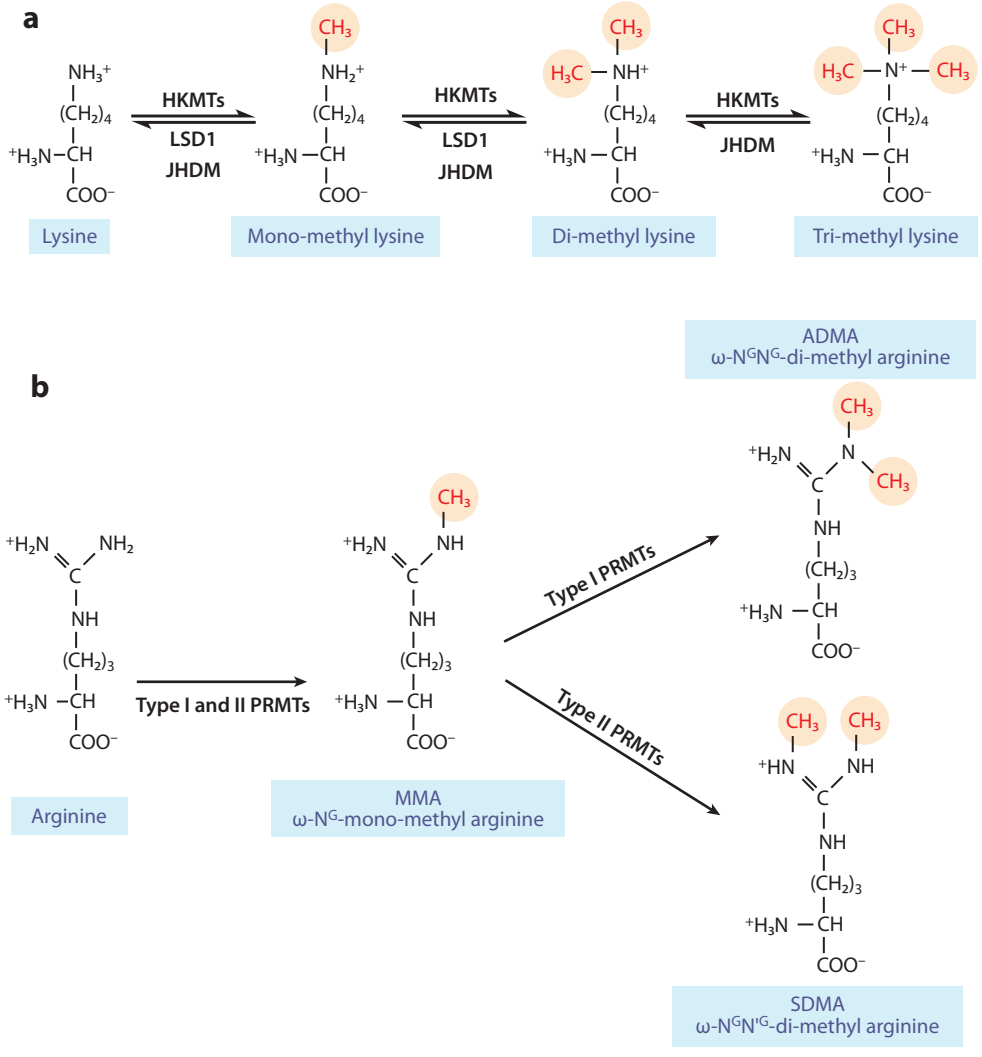


**Figure 2**

Histone methylation and demethylation on lysine or arginine residues produce a complex and dynamically regulated system of epigenetic marks. (*a*) Mono-, di-, and tri-methylation are produced by dynamic lysine methylation catalyzed by HKMTs (histone lysine methyltransferases) and histone demethylases: LSD1 (lysine-specific demethylase1) and JHDMs (JmjC domain–containing histone demethylases). (*b*) Arginine methylation by type I and type II PRMTs.

(11, 77). Unlike H3K9me2, H3K9me3 is enriched in euchromatin, where most active genes are found (87, 136).

KRYPTONITE (KYP) (48), also known as SU(VAR)3–9 homolog 4 (SUVH4) (85), was the first plant histone H3K9 methyltransferase identified. It was found in two independent genetic screens as a suppressor of *stabilized clark kent* (*clk-st*), a *superman* epiallele in which *SUPERMAN* (*SUP*) is silenced by DNA methylation, or of *PHOSPHORIBOSYLANTHRANILATE*

*ISOMERASE* (*PAI*). Both of these screens involved reactivation of loci that were transcriptionally silenced by DNA methylation, suggesting that H3K9 methylation plays a role in DNA methylation–mediated gene silencing (48, 85). KYP/SUVH4 lacks the N-terminal chromodomain (chromatin organization modifier domain) of SU(VAR)3–9 and instead has a YDG (named for three conserved amino acids)/SRA (SET and RING associated) domain for chromatin binding. KYP/SUVH4 is a bona fide writer of histone H3K9 methylation through its SET domain, similar to its homolog SU(VAR)3–9 (48). However, unlike SU(VAR)3–9, which can add one, two, or three methyl groups to H3K9, KYP/SUVH4 can add one or two, but not three, methyl groups to histone H3K9 in vitro (47). In *kyp/suvh4* nuclei, accumulation of H3K9me2 in heterochromatin foci was greatly reduced, but H3K9me1 was not significantly affected, revealing that KYP/SUVH4 is the major histone H3K9me2 methyltransferase. SUVH5 and SUVH6, two close KYP/SUVH4 homologs, were demonstrated to methylate H3K9 in vitro and are partially redundant with KYP/SUVH4 (32, 33, 47).

Silencing of *SUP* and *PAI* correlates with heavy DNA methylation, which is closely associated with histone H3K9me2 at heterochromatin (5, 75, 137). In the *Arabidopsis* genome, approximately 6% of cytosine nucleotides are methylated. Unlike the exclusive CG methylation in mammals, there are three major DNA methylation patterns in *Arabidopsis*: methylation occurs at 24% of the CGs, 6.7% of CHGs (H is A, C or T), and 1.7% of CHHs (25). De novo DNA methylation (methylation of an unmethylated template) in all sequence contexts (CG, CHG, and CHH) is mainly catalyzed by DOMAINS REARRANGED METHYLASE2 (DRM2), a homolog of mammalian de novo DNA methyltransferase DNMT3 (14, 16). Genes involved in the biogenesis or action of 24nt heterochromatin–associated siRNAs also affect de novo DNA methylation (19). Once the DNA methylation pattern is established, maintenance of methylation depends on the specific sequence context: CG methylation is exclusively maintained by cytosine-DNA-methyltransferase MET1, a mammal DNA methyltransferase DNMT1 homolog; non-CG methylation is redundantly controlled in a locus-specific manner by CHROMOMETHYLASE3 (CMT3), a plant-specific DNA methyltransferase, and DRM2 (15).

Characterization of the interplay between histone H3K9 methylation and DNA methylation reveals that H3K9 methylation is critical for maintenance of genomewide transcriptional gene silencing and genome stability (137). In the *kyp/suvh4* mutant, reduced H3K9me2 leads to loss of non-CG DNA methylation catalyzed by CMT3, thereby derepressing the silenced *SUP* and *PAI* and endogenous *TA3* transposon (48, 85). KYP/SUVH4 also acts at the SINE retroelement *AtSN1* (*Arabidopsis thaliana short interspersed element 1*), a region with high levels of CHH methylation, intermediate levels of CHG methylation, but only low levels of CG methylation (58). DNA methylation profiling reveals that CMT3 targets genomewide transposons and retrotransposons (133), and expression profiling of *kyp/suvh4* and *cmt3* identified hundreds of reactivated transposable elements as common targets of both KYP/SUVH4 and CMT3 (134). High-resolution genomewide ChIP-chip analysis further reveals a very high coincidence between H3K9me2 and CHG methylation (11). These results demonstrated that maintenance of non-CG DNA methylation requires histone H3K9 methylation and that these modifications are critical for repression of transposable elements, arrays of ribosomal RNA genes (rDNA), and other repetitive sequences.

Other lines of evidence suggest that DNA methylation also reinforces histone methylation in a positive feedback loop. For example, impaired CG DNA methylation in *met1* mutants is associated with decreased H3K9me2 at 180bp centromeric repeats, endogenous *Ta2* transposon, and other transposable elements (56, 87, 119, 129). Furthermore, loss of non-CG methylation in *drm1drm2cmt3* triple

mutants results in significant loss of H3K9me2 at *AtSN1*, whereas loss of *met1* will not erase histone H3K9me2 at *AtSN1* (58). It is noteworthy that histone H3K9 methylation patterns regulated by CG and non-CG methylation are locus specific and do not overlap. Two critical features for such locus specificity include the density of CG, CHG, and CHH sequences at each locus, and the presence of either inverted repeats or tandem repeats.

The mechanisms that tie histone methylation to DNA methylation have been partially elucidated by the identification of methylated DNA–binding proteins. For example, YDG/SRA domains of KYP/SUVH4 and SUVH6 preferentially bind methylated CHG DNA, suggesting a role of DNA methylation in recruiting H3K9 methyltransferases (58). Once recruited to the target region, the SET domains of these histone H3K9 methyltransferases methylate adjacent histones (**Figure 3**). VIM 1 (VARIANT IN METHYLATION 1), one of the YDG/SRA domain–containing proteins, preferentially binds to methylated CG and is required for decondensation of centromeric heterochromatin in interphase (141). Loss of *VIM1* and two homologs, *VIM2* and *VIM3*, leads to a dramatic loss of CG methylation, thereby releasing *FWA* gene silencing (140). VIM1 has been shown to interact with tobacco NtSET1, a homolog of KYP/SUVH4; also, inverted CCAAT box binding protein (ICBP90), a human homolog of VIM1, binds to methylated H3K9 and DNMT1 (12, 63, 79, 114, 117). These results suggest that VIM proteins coordinate the two histone modifiers, resulting in mutually reinforcing H3K9 methylation and CG DNA methylation in heterochromatin formation. Moreover, SUVH2 and SUVH9, two SU(VAR)3–9 homologs that also contain a SET domain and a YDG/SRA domain, were shown to be required for RNA-directed DNA methylation (RdDM) (59). The YDG/SRA domain of SUVH2 preferentially binds to methylated CG sites, while that of SUVH9 mainly binds to methylated CHH sites (59). Taken together, these findings suggest a self-reinforcing loop between H3K9me2 and DNA methylation in *Arabidopsis* (**Figure 3**).

In addition to YDG/SRA, methyl-CG-binding domain (MBD) proteins are evolutionarily conserved and represent another group of proteins that bind methylated DNA. In mammals, MeCP2 binds methylated CG and recruits diverse HKMTs, histone deacetylases, and chromatin remodeling factors to repress transcription of local chromatin. There are 13 MBDs in *Arabidopsis* and 17 MBDs in rice (**http://www.chromdb.org**) (38). AtMBD5, AtMBD6, and AtMBD7 bind methylated CG sites and preferentially target to chromocenters, probably recruited by DECREASE IN DNA METHYLATION 1 (DDM1), a SWI2/SNF2 chromatin remodeling protein (50, 110, 146). The study of MBDs is just emerging; for recent reviews see Reference 40.

SUVR4, which is closely related to G9a HKMT, requires a mono-methylated H3K9 peptide as substrate, whereas its two close homologs SUVR1 and SUVR2 do not have detectable methyltransferase activity (132). SUVR4 lacks the YDG/SRA domain found in the SUVH genes. Instead, SUVR4 contains a novel N-terminal domain and preferentially localizes to the nucleolus and noncondensed nuclear bodies, where it may function as a repressor of rDNA gene clusters and/or euchromatin (132). Although genetic data are lacking, the identification of SUVR4 as a novel type of H3K9 methyltransferase is the first step towards studying how H3K9me2 acts on euchromatin.

The rice genome is larger and contains more repetitive sequences (in over 40% of the genome) than the *Arabidopsis* genome. Unlike the highly enriched H3K9me2 within chromocenters in *Arabidopsis*, H3K9me2 in rice is distributed along the chromosomes, which is similar to many species with larger genome sizes (46). Rice SET DOMAIN GROUP PROTEIN 714 (SDG714) specifically methylates histone H3K9 and mainly localizes in heterochromatic regions, which is similar to KYP/SUVH4 (29, 30). *SDG714* RNAi plants (*SDG714IR*s) have decreased H3K9me1/2 at

**SDG:** SET domain group protein

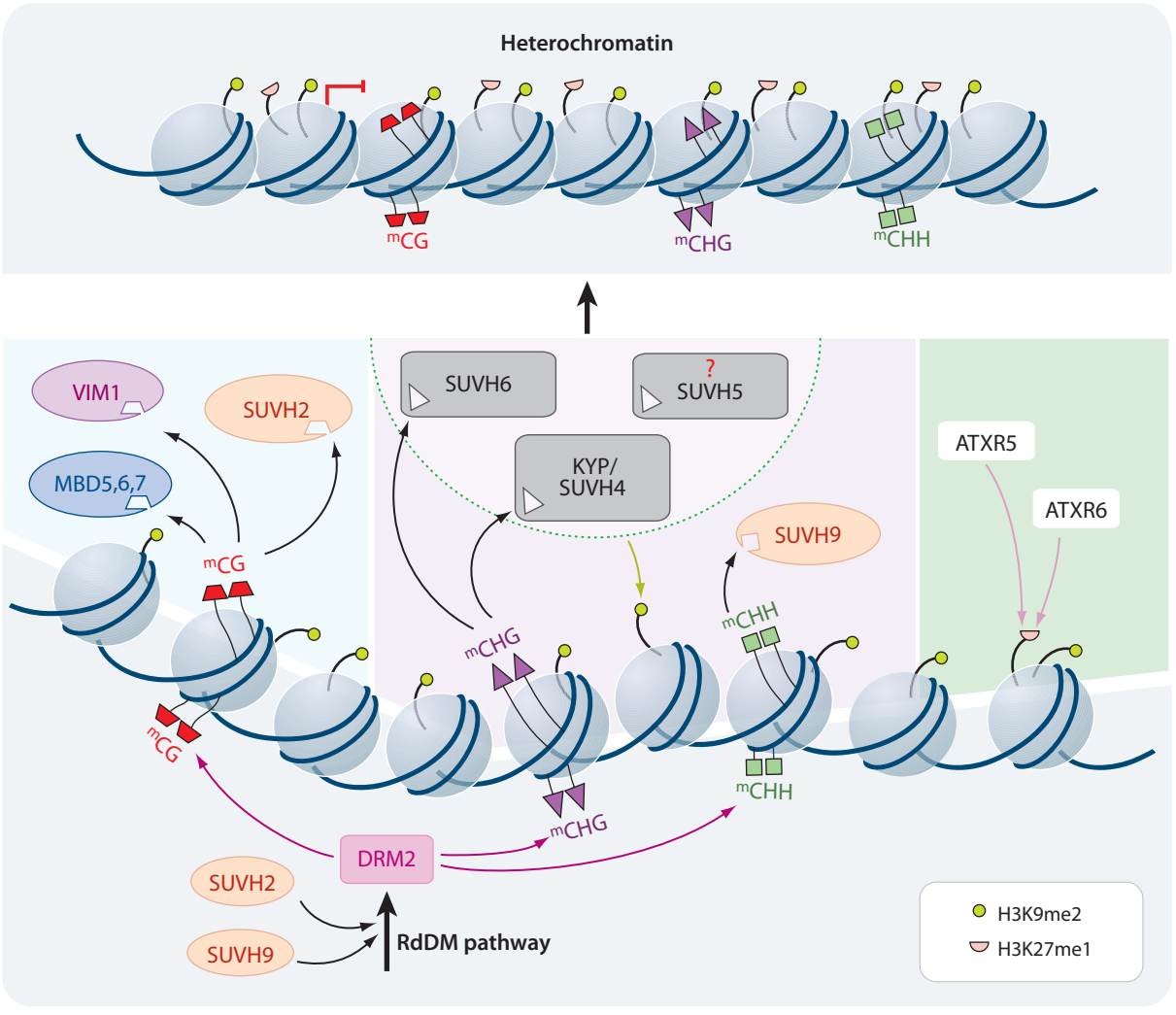


**Figure 3**

The H3K9me2 and H3K27me1 pathways control constitutive heterochromatin formation in parallel. The DNA methylation–dependent H3K9me2 pathway and the DNA methylation–independent H3K27me1 pathway have been proposed based on genetic and biochemical evidence. In the H3K9me2 pathway, DRM2 initiates de novo DNA methylation at all CG (*red trapezoids*), CHG (*purple triangles*), and CHH (*green boxes*) sites. Methylated CG nucleotides are recognized and bound by different classes of methylated DNA-binding proteins. In contrast to SUVH9, which binds to the methylated CHH, the YDG/SRA domains of the histone H3K9 methyltransferases are recruited to the methylated CHG target regions, and the SET domains of these enzymes methylate adjacent histones to form a self-reinforcing loop between H3K9me2 and CHG DNA methylation. The H3K27me1 mark is set by histone methyltransferases ATXR5 and ATXR6. Thus constitutive heterochromatin is maintained by two pathways in parallel.

retrotransposon *TOS17*, coinciding with decreased DNA methylation. Most important, transcription was reactivated, resulting in transposition of *TOS17* in *SDG714IR*s. This shows that histone H3K9 methylation is critical for maintenance of genome stability (30).

## The Impact of Histone H3K9 Methylation on Plant Development

Although histone H3K9 methylation is essential for gene silencing, the *kyp/suvh4 suvh5 suvh6* triple mutant does not show obvious

developmental defects. In addition, loss-of-function of *SUVH2* shows no obvious phenotypes (94). Only when combined with *suvh2 suvh9* did *suvh4* show pleiotropic developmental defects, including curled leaves and short stature, which are also seen in the *drm1 drm2 cmt3* triple mutant (15, 59). These phenotypes are caused by derepression of *SUPPRESSOR of drm1 drm2 cmt3* (*SDC*), an F-box gene which is silenced by a combination of the RNA-directed DNA methylation (RdDM) pathway and CMT3 in wild type (45). NRPE1/NRPD1b/DRD3 and NRPE2/NRPD2a/DRD2 are the largest and the second largest subunits of the plant-specific RNA polymerase V (62), which serves to guide the ARGONAUTE4 (AGO4)–mediated RdDM pathway (104). Any combination of mutants that blocks both the CMT3 pathway and the RdDM pathway shows the same development phenotype (e.g., *drm1 drm2 kyp*, *nrpd2a nrpd2b kyp* and *nrpd2a nrpd2b cmt3*) (45, 59). This phenotype can also be produced by the ectopic expression of *SDC*, an example of developmental gene regulation mediated by H3K9me2 involved in the non-CG methylation pathway.

## Dual Roles of H3K27 Methylation in Heterochromatin Formation and Plant Development

Histone H3K9 and H3K27 methylation are two repressive marks found in both animals and higher plants. Similar to H3K9, H3K27 can be mono-, di-, or tri-methylated in *Arabidopsis*. In addition, both H3K27me3 and H3K9me3 are localized in euchromatin, but these two marks do not show significant overlap (136). H3K9me1/2 and H3K27me1 are enriched at constitutive silenced heterochromatin in *Arabidopsis* (36, 87). However, unlike H3K9me2, which is strongly associated with DNA methylation, H3K27me1 is independent of DNA methylation (49, 87), which suggests that their deposition, maintenance, and perhaps function are mediated by distinct mechanisms.

ARABIDOPSIS TRITHORAX-RELATED PROTEIN 5 (ATXR5) and ATXR6 are the only enzymes proved biochemically to mono-methylate H3K27 (49). The *atxr5 atxr6* double mutant shows partial disruption of constitutive heterochromatin, reduced H3K27me1 at chromocenters, and reactivated silenced genes such as *Ta3* and other heterochromatin markers, indicating that both H3K27me1 and H3K9me2 act at common loci. Interestingly, the *atxr5 atxr6* mutant did not affect DNA methylation or global distribution of H3K9me2; also, H3K27me1 at chromocenters was not affected in the *kyp suvh5 suvh6* triple mutant (49). These results suggest that both the DNA methylation–dependent H3K9me2 pathway and DNA methylation–independent H3K27me1 pathway control constitutive heterochromatin formation in parallel (**Figure 3**). Although the molecular mechanism of triggering or maintaining H3K27me1 at constitutive heterochromatin is not fully understood, the identification of ATXR5 and ATXR6 as novel H3K27me1 methyltransferases is a step forward in resolving this mystery.

H3K27me3 has been implicated in developmental regulation since it provides a cellular memory to maintain the repressed transcriptional states of targets genes during cell division. In animals, E(Z), a SET domain histone methyltransferase within polycomb repressive complex 2 (PRC2), catalyzes tri-methylation of H3K27, which in turn is recognized by the chromodomain of POLYCOMB (Pc), a core component of the PRC1 complex. The *Arabidopsis* genome encodes homologs of all members of the conserved PRC2 complex, including three E(Z) homologs [CURLY LEAF (CLF), MEDEA (MEA), and SWINGER (SWN)], three Su(z)12 homologs [FERTILIZATION-INDEPENDENT SEED2 (FIS2), EMBRYONIC FLOWER2 (EMF2), and VERNALIZATION2 (VRN2)], five p55 homologs [MULTICOPY SUPPRESSOR OF IRA (MSI)1–5], and only one homolog of Esc [FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)] (66, 103). Although CLF, MEA, and SWN are widely believed to be H3K27me3 methyltransferases, direct biochemical evidence is absent, possibly due to

the requirement for the whole PRC2 complex for methyltransferase activity to occur.

Genomewide profiling of H3K27me3 reveals distinct establishment and spreading mechanisms between plants and animals (149). Unlike animals, wherein H3K27me3 associates with low-nucleosome-density regions and forms a continuous block of inactive chromatin, the H3K27me3 modification in *Arabidopsis* preferentially localizes to the transcribed regions of genes, with a remarkable bias towards those immediately upstream of promoters and the 5′ end of transcribed regions of genes, consistent with a role in transcriptional repression (136, 149). In addition, a large number of genes (~17% of the coding genes) was found to be marked with H3K27me3, indicating that H3K27me3 is a major gene silencing mechanism in *Arabidopsis* (149). In this case, even with various possible subunit combinations for functional PRC2, such a high number of H3K27me3 target sites indicates that each PRC2 complex must be targeted to multiple sites and/or that more components beyond the four core members are required to regulate the expression of targets. Identification of novel proteins involved in targeting H3K27me3 to a specific locus for gene repression is of great interest.

In *Arabidopsis*, at least three distinct PRC2 core complexes regulate common target genes with specific functions, including regulating cell proliferation and differentiation and controlling developmental phase transition upon environmental changes. For recent reviews see References 66, 103.

The first PRC2 complex found in *Arabidopsis* is the FIS-containing complex, which is composed of MEA/FIS1(41), FIS2 (82), FIE/FIS3 (21, 98), and MSI1 (65). These four proteins have been characterized in studies of mutants that exhibit a gametophytic maternal effect: Self-fertilized heterozygous plants produce 50% aborted seeds, which can be observed only when the mutation is inherited through the female gametophyte. In addition, mutation in any of the FIS-containing PRC2 complex components leads to endosperm development in the absence of fertilization, suggesting that this complex is required for preventing cell proliferation in seed development. Biochemical assays further verified that MEA physically interacts with FIS2 and FIE, and FIE interacts with MSI and MEA, but no interaction between MSI1 and FIS2 has been demonstrated (65, 121, 138). Interestingly, a few genes, including *FIS2* and *MEA* in the PRC2 complex, are subject to imprinting in *Arabidopsis*. DNA demethylation, mediated by the DNA glycosylase DEMETER (DME), Retinoblastoma (Rb), and Rb-associated protein 48 (RbAp48), is required for *FIS* paternal imprinting (24, 61). FIS-containing complex silences *MEA* transcription throughout vegetative life and male gametogenesis. In the endosperm, the maternal allele of *MEA* is expressed and forms an essential component of a PcG complex, which maintains silencing of the paternal *MEA* allele. This feedback loop ensures a complete maternal contribution to *MEA* expression (60).

The EMF2-containing PRC2 complex, which is composed of EMF2, CLF, FIE, and MSI1, has been identified based on its function in repressing floral transition and floral homeotic gene expression. Plants defective in *EMF2* germinate directly into a small, sterile inflorescence with a terminal flower. Since *emf2* mutants bypass vegetative growth, the wild-type EMF2 must be required for repression of precocious reproductive growth (128). Consistently, in *emf2* mutants, the floral organ identity genes, such as *APETALA1* (*AP1*), *AP3*, *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*), are ectopically expressed (92), and H3K27me3 of the *AG* locus is dramatically decreased (13). Mutations in *CLF* result in early flowering and pleiotropic phenotypes, including curled leaves and partial homeotic transformation of the sepals and petals to carpels and stamens, respectively (39, 113). The transcriptional derepression of *AG* and the MADS-box gene *AGAMOUS–LIKE 19* (*AGL19*) in *clf* mutants could partially account for the abnormal phenotypes (112). In addition, recent studies have demonstrated that EMF2, CLF, and FIE are also necessary for repressing the expression

of the floral repressor *FLC* and the florigen gene *FLOWERING LOCUS T* (*FT*) through directly binding *FLC* and *FT* chromatin (53). Consistent with these observations, H3K27me3 modification of *AG* (113), *AGL19* (112), *FLC*, and *FT* (53) loci is decreased in *clf* mutants. A missense mutation within the SET domain of *CLF* results in a mutant phenotype similar to a *clf* null allele, indicating that CLF could be a bona fide HKMT in vivo and the enzymatic activity is necessary for its biological function (113). The E(Z) homolog SWN exhibits partial functional redundancy with CLF and MEA: *swn* mutants have no obvious phenotypes but strongly enhance the phenotypes of *clf* and *mea* mutants in *clf swn* and *mea swn* double mutants (20, 84, 138). In addition, by immunostaining experiments, H3K27me1 level was not affected in *clf swn* double mutants, but the staining intensity of H3K27me2/3 in euchromatin was reduced, further suggesting that CLF and SWN act on H3K27me2/3 in the euchromatin (76).

The third, VRN2-containing PRC2 complex contains three additional PHD finger proteins, namely, VERNALIZATION INSENSITIVE 3 (VIN3), VERNALIZATION 5/VIN3 LIKE 1 (VRN5/VIL1, a partner of VIN3), and VEL1/VIL2, in addition to the four core subunits, VRN2, FIE, CLF/SWN, and MSI1 (20, 27, 142). The VRN2-containing PRC2 complex is implicated in vernalization, a process whereby flowering is promoted by exposure to cold in winter-annual *Arabidopsis* accessions (44).

Vernalization is one of the best characterized examples of epigenetic regulation induced by environmental signals. Flowering time variation in nature is mostly contributed by the *FRIGIDA* (*FRI*) and *FLC* loci. FLC, a MADS-box protein, plays a central role in repressing flowering (90, 115). FRIGIDA (FRI), a coiled-coil domain protein, suppresses flowering by elevating the expression of *FLC* in winter-annual plants (55) and *FLC* expression could be repressed by vernalization to promote flowering in the spring (28, 125). The repressed status of *FLC* is maintained by VRN2-containing PRC2 complexes (27, 142) in the warm conditions during plant development until it is reset during the formation of gametes or during embryo development (23, 116).

VRN2 constitutively associates with the entire *FLC* locus in both nonvernalized and vernalized plants (27). In nonvernalized seedlings, *FLC* expression is not repressed even in the presence of VRN2. Prolonged cold induces VIN3 expression (124). VIN3, VRN5/VIL1, and VEL1/VIL2 are recruited to *FLC* chromatin and form a large VRN2-PRC2 complex together with the four core members to mediate the deposition of H3K27me3 (27, 124). Furthermore, VIN3 is involved in deacetylation of the *FLC* locus since the vernalization-induced acetylation changes do not occur in *vin3* mutants (124). *FLC* repression does not occur until *VIN3* is induced; therefore, VIN3 plays a key role in the initial establishment of the repressive state of *FLC* (124). After subsequent return to the warm conditions, VIN3 expression is quickly shut down, while the VRN5/VIL1 and VEL1/VIL2 containing VRN2-PRC2 complex mediates the spread of H3K27me3 across the *FLC* locus to maintain a mitotically stable repressed state of *FLC* (6, 27, 35, 124, 127) (**Figure 4**). These data suggest that vernalization-induced epigenetic silencing of *FLC* involves dynamic association and changing composition of polycomb complexes at different stages in response to environmental changes (27).

It is believed that the H3K27me3 marks at *FLC* chromatin recruit LIKE HETEROCHROMATIN PROTEIN 1 [LHP1; also known as TERMINAL FLOWER 2 (TFL2)] which specifically interacts with H3K27me3 to fully silence *FLC* upon return to warm conditions (37, 93, 126, 136, 150). In addition, a DNA-binding protein, VRN1, directly binds *FLC* chromatin and maintains the silenced state of *FLC*, although the biochemical role of VRN1 is still unknown (71). VIN3, VRN2, and VRN1 are also required for H3K9 methylation in silencing *FLC* expression (6, 124) (**Figure 4**). But it is not clear which HKMT is responsible for catalyzing H3K9 methylation in *FLC* chromatin during vernalization, because single

**LHP1:** LIKE HETEROCHROMATIN PROTEIN 1

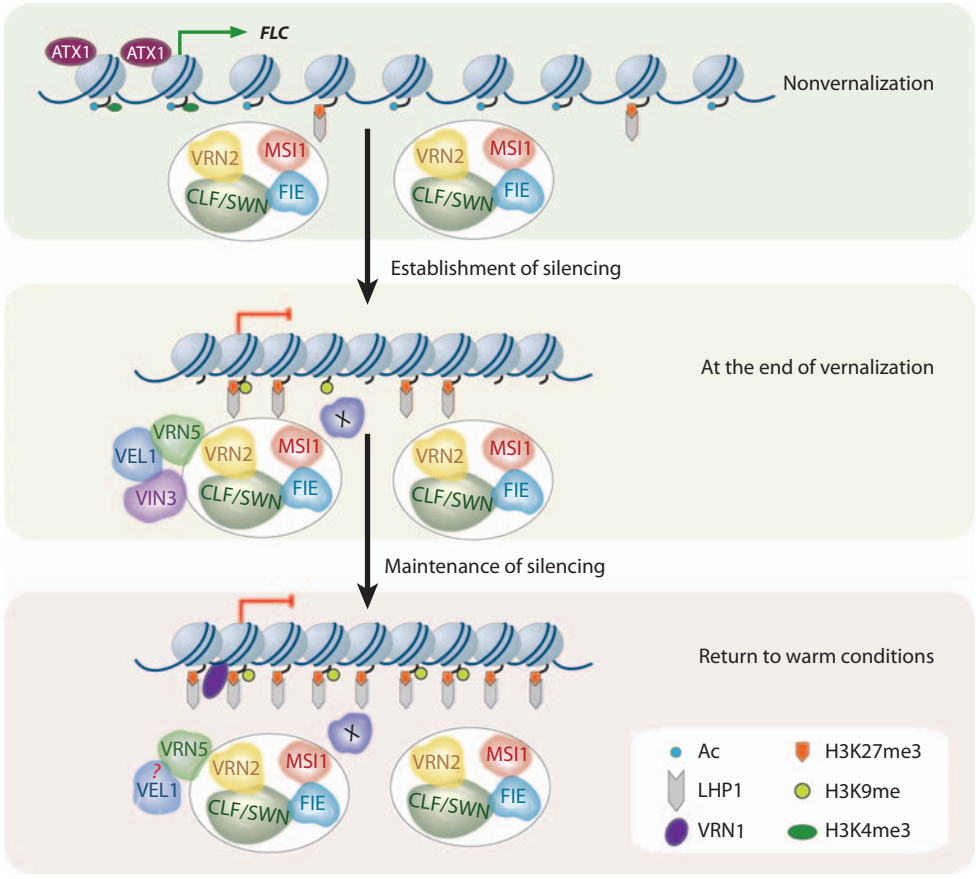


**Figure 4**

Model for vernalization-mediated histone modifications of *FLC* chromatin in winter-annual accessions. Before vernalization, H3K4me3 created by ATX1 and histone acetylation promote *FLC* transcription despite the presence of the low level of repressive mark H3K27me3 catalyzed by the VRN2-PRC2 complex. Vernalization induces VIN3 expression; VRN5 and VEL1 are recruited to *FLC* chromatin to form a larger VRN2-containing PRC2 complex together with PRC2 core members to mediate H3K27me3 and histone deacetylation; and the silencing state is established and maintained. Furthermore, one or multiple unknown H3K9 methyltransferase(s) (denoted by an X) is required for catalyzing H3K9 methylation. Subsequently, LHP1 and VRN1 are recruited to stably maintain the repressed state of *FLC*.

mutants of several known and putative H3K9 HKMTs (8 *suvh* and 4 *suvr*) are not impaired in repressing *FLC* in response to vernalization (93). One possible explanation is functional redundancy among the H3K9 methyltransferases.

Unlike the well-characterized *Arabidopsis* E(Z) homologs, little is known in rice. The rice genome encodes two E(Z) homologs: OsiEZ1/OsSET1 and OsCLF (83). OsiEZ1/OsSET1 localizes in the nucleus, and overexpression of its SET domain in *Arabidopsis* alters shoot development at early developmental stages (74, 131). Obviously, further studies on PRC2 complexes are necessary to reveal the roles of histone H3K27 methylation in rice development.

## H3K4 Methylation and Trithorax Group Proteins

Histone H3K4 methylation is mediated by Trithorax group (TrxG) proteins, which act

antagonistically to PcG proteins in regulating the homeotic gene (*HOX* gene) expression in animals. The functions of TrxG and PcG proteins are conserved in plants and animals, where PcG proteins are generally required for maintaining a repressive state and TrxG proteins are responsible for the maintenance of an active state (3, 103). ARABIDOPSIS TRITHORAX 1 (ATX1), a homolog of histone H3K4 methyltransferase TRX, is an active histone methyltransferase specific for histone H3K4 (2). Mutations in *ATX1* lead to abnormal floral organ identity and slightly early flowering (2, 102). ATX1 directly interacts with the promoter and the first exon of *FLC* locus and catalyzes H3K4me3 modification (**Figure 4**) (102). Consistent with ATX1 being an H3K4me3 methyltransferase, H3K4me3 at *FLC* was reduced but K3K4me2 remained unchanged in the *atx1* mutants (102).

*atx1* mutants have reductions of 6–8% in H3K4me2 and ~15% in H3K4me3 at a global level, indicating that some other HKMTs could also catalyze H3K4 methylation (1). Indeed, the *Arabidopsis* genome encodes five TRX homologs and seven TRX related proteins, with ATX2, a paralog of ATX1 arising from a chromosomal duplication (7, 108). However, mutations in *ATX2* have no visible defects and the expression patterns of ATX1 and ATX2 are different. Furthermore, ATX1 is mainly required for H3K4me3, whereas ATX2 is responsible for H3K4me2, suggesting that they possess divergent functions (108).

The genomic distribution patterns of methylated H3K4 have revealed similar localization patterns in *Arabidopsis* and rice (73, 77, 148). H3K4me1/2/3 are highly enriched in genic regions but depleted in transposons. On the genomic scale, levels of H3K4me3, not H3K4me1/2, are positively correlated with transcript abundance in *Arabidopsis* and rice. Consistently, H3K4me1/2 occur on both active and inactive genes, whereas H3K4me3 is present exclusively on active genes in *Arabidopsis* and rice (73, 148). H3K4me1 is enriched in the gene body regions with an apparent 3′ bias in *Arabidopsis* (148), whereas both H3K4me2/3 modifications are enriched in the promoters and the 5′ end of transcribed regions with H3K4me3 peaking slightly upstream to H3K4me2 in *Arabidopsis* and rice (73, 148). These data suggest that the distribution patterns of H3K4 methylation are generally consistent with their functions.

## H3K36 Methylation and ASH1 Group Proteins

Histone H3K36 is specifically methylated by histone methyltransferase ASH1 in mammals and *Drosophila*, and H3K36me2/3 are linked to transcription elongation (10, 72). The *Arabidopsis* genome encodes at least four ASH1 homologs and three ASH1-related proteins (7). Among them, the methyltransferase activities of SDG8, SDG26, and SDG4 have been identified in vitro or in vivo (17, 143, 153). SDG8 and SDG26 are nuclear proteins with histone methyltransferase activities in vitro on oligonucleosomes, but not on mono-nucleosome and free histones (143). SDG8 is the major H3K36 methyltransferase in vivo required for global H3K36me2/3 (31, 143). Mutations in *SDG8* and *SDG26* cause early- and late-flowering phenotypes, respectively (143, 153). SDG8 activity is specific to H3K36me2/3, to activate the expression of *FLC* and *MADS AFFECTING FLOWERING* (*MAF*) genes, whereas SDG26 represses the same group of genes possibly in an indirect manner (143). Besides early flowering, the *sdg8* mutants show increased shoot branching and altered carotenoid composition, indicating broader roles of SDG8 in regulating other developmental or physiological processes (18, 31). *SDG4* encodes an ASH1-related, chromosome-associated protein highly expressed in the pollen. In *sdg4* pollen, the levels of H3K4me2 and H3K36me3 were greatly reduced in the vegetative nucleus and the length of the pollen tube was reduced, indicating that SDG4 controls pollen tube growth possibly by regulating the expressions of some pollen-specific genes via histone methylation (17).

PRMT: protein arginine methyltransferase

Although the important role of H3K36 methylation is emerging in *Arabidopsis*, it is not clear whether the mechanism of such modifications in the regulation of transcription is similar across different kingdoms. In animals and yeast, histone H3K36me2/3 act as docking sites to recruit other histone modifiers, such as deacetylases (HDACs), during late elongation to ensure the fidelity of transcription. H3K36 methylation also recruits histone acetyltransferase for transcription activation, and such opposite action reflects the complexity of transcriptional elongation (72). In addition, new evidence from *Caenorhabditis elegans* reveals that H3K36me3 differentially covers the exons over introns of actively transcribed genes, thus providing epigenetic information for a splicing-related marking mechanism (67). Given the fact that SDG8 and SDG26 confer similar enzymatic activities and specificities, but loss-of-function mutants display opposite phenotypes, it will be interesting to understand how each H3K36 methyltransferase is targeted to distinct loci and how H3K36 methylation functions in transcription regulation.

## ARGININE METHYLATION BY PRMTS

Protein arginine methylation plays an essential role in regulating transcription, RNA processing, nuclear transport, DNA-damage repair, and signal transduction (9). Arginine methylation mainly occurs at Arg2 (R2), Arg8 (R8), Arg17 (R17), Arg26 (R26) of histone H3, and Arg3 (R3) of histone H4 and is catalyzed by a small group of protein arginine methyltransferases (PRMTs). PRMTs fall into four biochemical classes, among which type I and type II enzymes are the most important and best characterized (9). These two types of PRMTs catalyze mono-methylation of Arg (MMA; a single methyl group added on the terminal nitrogen atom yielding ω-$N^G$-monomethyl arginine) as an intermediate. Type I PRMTs then form asymmetric di-methylated Arg (ADMA; two methyl groups added on the same nitrogen atom of the guanidine group forming ω-$N^G$,$N^G$-di-methyl arginine), whereas type II PRMTs yield symmetric di-methylation of Arg (SDMA; two methyl groups placed on two different guanidino nitrogens producing ω-$N^G$,$N'^G$-di-methyl arginine) (**Figure 2*b***) (8). In mammals, PRMT1 and PRMT5 represent the main type I and type II methyltransferase activities that mono- and di-methylate histone H4R3 asymmetrically or symmetrically, respectively. In addition to PRMT1, PRMT4/Coactivator Associated Arginine Methyltransferase 1 (CARM1) is also a very important type I methyltransferase that di-methylates asymmetrically R2, R17, and R26 in histone H3. Embryos of *prmt1* knockout mice die soon after implantation and *CARM1*-null mice die just after birth, indicating that PRMT1 and CARM1 play essential roles in development (8).

In *Arabidopsis*, nine PRMTs are present in the genome (95). AtPRMT4a and AtPRMT4b, which are homologs of human CARM1, asymmetrically di-methylate histone H3 at R2, R17 (H3R17me2a), and R26 in vitro and are required for H3R17me2a in vivo (**Table 1**). AtPRMT4a and AtPRMT4b are functionally redundant and only *atprmt4a atprmt4b* double mutants show an *FLC*-dependent late-flowering phenotype (96).

H4R3 can be di-methylated symmetrically by AtPRMT5/SKB1 (100, 111, 139) as well as asymmetrically by AtPRMT10 (95), AtPRMT1a, and AtPRMT1b (144). Interestingly, although AtPRMT4a, AtPRMT4b, AtPRMT5/SKB1, and AtPRMT10 confer distinct enzymatic specificities, all of them are involved in regulating flowering time (95, 96, 100, 111, 139). The expression of *FLC* is increased in all *atprmt4a atprmt4b* double, *atprmt5/skb1*, and *atprmt10* mutants. Moreover, the *atprmt5 atprmt10* double mutant displays an additive effect on flowering time and *FLC* expression, indicating that the mechanisms of regulating flowering by these two PRMTs are different (95). In addition to late flowering, *atprmt5* mutants also show other pleiotropic phenotypes, including growth retardation and dark green and curly leaves, suggesting

that AtPRMT5/SKB1 plays important roles in many developmental processes (100, 111, 139). Surprisingly, unlike the embryo lethality of *prmt1* knockout mice, double mutants of *atprmt1a atprmt1b* have no obvious phenotypes (Y. Zhang and X. Cao, unpublished data). One possible explanation is the functional redundancy between AtPRMT1a, AtPRMT1b and other members of the AtPRMT family.

Because of functional redundancy of AtPRMTs, it is possible that in addition to flowering time regulation, AtPRMTs are also involved in regulating many other developmental processes. Therefore, the full spectrum of the developmental functions of AtPRMTs remains to be uncovered. A wide variety of nonhistone proteins have been identified as the substrates of PRMTs in animals. For instance, PRMT4/CARM1 has been shown to methylate histone acetyltransferases p300/CBP (22) and PRMT5 methylates small nuclear ribonucleoproteins (snRNPs) SmD1 and SmD3 (88). Therefore, identification of more in vivo substrates of AtPRMTs will help to elucidate the molecular mechanisms underlying how arginine modification contributes to regulation of gene expression.

## READERS OF HISTONE METHYLATION

Histone methylation serves as an important epigenetic mark for the recruitment of specific effector proteins that regulate nuclear processes, such as gene transcription (10). In animals, the chromodomain of HP1 was first identified to specifically bind histone H3K9me3 (4, 68). Currently, the known effectors of methylated histone are classified into three superfamilies: first, effectors containing chromolike domains of the royal superfamily [including chromodomain, tudor domain, malignant brain tumor (MBT), PWWP (PWWP is named after a conserved Pro-Trp-Trp-Pro motif), and plant Agenet module]; second, the plant homeodomain finger (PHD) superfamily; and third, effectors containing the WD40 repeat in WDR5 (106, 130). Human WDR5 recognizes unmodified, mono-, di-, and tri-methylated H3K4 peptides with a stronger affinity for H3K4me2 and functions to present the lysine to an HKMT complex for further methylation. Recent structural and biophysical studies of WDR5 have presented a more complicated and controversial picture regarding methylation binding preferences, in which WDR5 recognizes unmethylated arginine in the H3 Arg2 context. For details, please see the recent reviews (106, 130). It is noteworthy that some readers recognize unmodified histone and the binding capacity is lost upon modification. For example, the PHD finger of BHC80 binds to unmodified H3K4 and recruits histone demethylase KDM1/LSD1 in mammalian cells. This binding is abolished when H3K4 is methylated (69).

**Effector:** a binding domain that recognizes and binds specifically to a posttranslationally modified protein (e.g., Chromodomain or PHD finger domain)

In *Arabidopsis*, a few readers, such as LHP1/TFL2, ORC1, AtING, AL, and WDR5a, have been shown to bind methylated histone in vivo or in vitro. LHP1/TFL2 is the only heterochromatin protein 1 (HP1) homolog with similar motifs to HP1: the chromodomain and the chromo shadow domain (37). In contrast to HP1, which recognizes and binds the H3K9me3 methylation in animals, genomewide analyses have demonstrated that LHP1/TFL2 functions similarly to Pc (a subunit of PRC1 in animals), which binds and colocalizes with the PRC2-mediated H3K27me3 modification (136, 150). In addition, the chromodomain of LHP1/TFL2 is necessary for binding of H3K27me3 at its target loci (34) and LHP1/TFL2 binding is required for stable silencing of H3K27me3-marked genes, such as *FLC* (93).

ORC1, the large subunit of the origin-recognition complex (ORC), was originally identified as a component of DNA replication initiation complexes. *Arabidopsis* ORC1a and ORC1b contain a PHD finger domain that is absent in yeast and animal ORC1. *Arabidopsis* ORC1 can interact with the H3K4me3 mark by the PHD finger domain, which is necessary for activating the transcription of target genes

JMJ: JmjC domain–containing protein

(26). *Arabidopsis* WDR5 homolog WDR5a is enriched at the *FLC* locus in the presence of FRI. Similarly to its counterpart in humans, recombinant WDR5a binds H3K4me2 peptides. *FRI* specifically mediates this binding in vivo, thereby increasing H3K4me3 and thus upregulating *FLC* expression to inhibit flowering (52). Recently, Lee and colleagues predicted 83 proteins containing canonical PHD fingers from the *Arabidopsis* proteome database and identified two groups of nuclear proteins containing PHD finger, AtING and AL, as readers that interact with H3K4me3/2 in vitro (70).

It is not fully understood how reader proteins translate the histone marks to direct downstream functions. Lines of evidence from animal studies show that some of the known readers are the subunits of protein complexes with chromatin remodeling or modification activities (130). For example, a PHD finger polypeptide Yng1p, a subunit of nucleosomal acetyltransferase of histone H3 (NuA3) complex, interacts specifically with H3K4me3 and recruits the NuA3 complex to promote downstream acetylation and transcriptional events (130). Moreover, several readers are histone modifiers. For instance, an H3K4 methylation reader protein JMJD2A is a histone demethylase (130) and DNA methyltransferase DNMT3A binds specifically to symmetric H4R3me2 created by PRMT5 (151). Future challenges in histone modification include the identification of more readers of histone modifications and understanding the mechanisms by which these effector proteins interpret the code of histone modifications to yield specific biological readouts.

## ERASERS OF HISTONE METHYLATION

Histone methylation is dynamically regulated by the writers and erasers. Histone demethylases play vital roles in regulating histone methylation homeostasis. Two types of demethylases with distinct mechanisms, amine oxidation by lysine-specific demethylase1 (LSD1) and hydroxylation by Jumonji C (JmjC) domain–containing proteins, remove methyl groups from methylated lysine residues (118, 135). In addition, these two groups of proteins use different cofactors and act on different substrates (**Figure 2*a***): Flavin adenine dinucleotide (FAD)-dependent KDM1/LSD1 acts only on di- and mono-methylated but not tri-methylated lysines, and a large family of JmjC domain–containing proteins using Fe(II) and α-ketoglutarate (αKG) as cofactors has demethylase activities towards tri-, di-, and mono-methylated lysines (64).

### KDM1/LSD1-Like Histone Demethylases

In *Arabidopsis*, there are four KDM1/LSD1 homologs, FLOWERING LOCUS D (FLD), LSD1-LIKE 1 (LDL1), LDL2, and LDL3. Among them, LDL1 was shown to demethylate di- and mono-methylated H3K4 (120). FLD, LDL1, and LDL2 contain conserved motifs and all are required for repressing *FLC* expression (43, 54). Consistent with its function in H3K4 demethylation, H3K4me2 was elevated in the *FLC* locus in *ldl1 ldl2* and *fld* mutants (54, 78). Also, in the *ldl1 ldl2* double mutant, H3K4me2 was elevated at the *FWA* locus, resulting in derepression of *FWA* in vegetative tissue (54). In addition to H3K4 demethylase activity, KDM1/LSD1 is involved in removing H3K9 methylation in mammalian cells upon cofactor binding, but it has not been confirmed in vitro yet (89). Therefore, studies of the biochemical properties of KDM1/LSD1-like enzymes will provide insight into the functions of this group of enzymes in histone methylation homeostasis.

### JmjC Domain–Containing Histone Demethylases

*Arabidopsis* and rice contain 21 and 20 JmjC domain–containing proteins (JMJs), respectively. These JMJs are grouped into five

subfamilies according to sequence similarities, including: the KDM5/JARID1 group, KDM4/JHDM3 group, KDM3/JHDM2 group, JMJD6 group, and JmjC domain–only group. Potential histone demethylases in *Arabidopsis* and rice have been predicted based on conservation of cofactor-binding amino acids (81).

*Increase in Bonsai Methylation 1* (*IBM1/JMJ25*) is essential for protecting active genes from ectopic H3K9me2 and CHG DNA methylation (91, 109). As a homolog of human KDM3/JHDM2, IBM1/JMJ25 has been predicted to be a histone H3K9me2, 1 demethylase (81, 109). Consistent with this prediction, the phenotypes of *ibm1* can be completely suppressed by mutations of *KYP/SUVH4* (109). Genomewide DNA methylation analysis in *ibm1* has revealed CHG hypermethylation in thousands of genes (91). And the CHG hypermethylation upon loss of *IBM1* still occurs in *drm2*, *rdr2* (*RNA-dependent RNA polymerase 2*), *ago4*, *nrpd1a* (the largest subunit of the plant-specific RNA polymerase IV), and *rdr6* mutants, indicating that the de novo ectopic deposition of CHG methylation in the *ibm1* mutant relies on a novel pathway that is independent of RNA-directed de novo DNA methylation (91). Moreover, mutation of *CMT3* completely suppressed the *ibm1* phenotype (109). These make CMT3 a good candidate for the de novo methyltransferase for loci hypermethylated in *ibm1* mutants. These findings furthered our insight into the interplay between DNA de novo methylation and H3K9 methylation. However, biochemical evidence of the IBM1/JMJ25 and other JMJs in plants as H3K9 demethylases is still lacking.

*Early Flowering 6* (*ELF6/JMJ11*) and its close homolog *Relative of Early Flowering 6* (*REF6/JMJ12*) belong to the KDM4/JHDM3 group (81, 97). The mammalian homolog, KDM4/JHDM3, was capable of reversing tri- and di-methylated histone H3K9 and H3K36 (64). REF6/JMJ12 and ELF6/JMJ11 play divergent roles in controlling flowering time, in which the *elf6/jmj11* mutant is early flowering and the *ref6/jmj12* mutant shows an *FLC*-dependent late-flowering phenotype (97). A recent study also demonstrated cooperative roles of ELF6/JMJ11 and REF6/JMJ12 in modulating brassinosteroid signaling (145). REF6/JMJ12 and ELF6/JMJ11 were recruited to BES1 target sites by physically interacting with BES1 and coincided with reduction of H3K9me3, indicating that REF6/JMJ12 might be capable of demethylating H3K9me3 (145). However, the biochemical properties of ELF6/JMJ11 and REF6/JMJ12 remain to be elucidated.

Rice JMJ706 also belongs to the KDM4/JHDM3 group and has been shown to demethylate histone H3K9me3 both in vitro and in vivo (123). Mutations in *JMJ706* lead to flower organ number variation of spikelets and global elevation of H3K9me3/2 (123).

*Maternal Effect Embryo arrest 27* (*MEE27/JMJ15*) was recovered in a genetic screen aimed at isolating mutants defective in female gametophyte development (99). MEE27/JMJ15 belongs to the KDM5/JARID1 group and was predicted to be an H3K4 demethylase (81). We developed an in vivo assay that detects global histone methylation changes upon overexpression of FLAG-tagged MEE27/JMJ15. We found that MEE27/JMJ15 is indeed a histone H3K4 demethylase that is capable of reversing H3K4me3, H3K4me2, and H3K4me1 in vivo (**Figure 5** and **Table 1**) (F. Lu, X. Cui, and X. Cao, unpublished data). A similar study also shows that JMJ14 is a histone H3K4 demethylase and regulates flowering time in *Arabidopsis* (80).

KDM6/JMJD3 and KDM2/JHDM1 group proteins demethylate H3K27me3/2 and H3K36me2/1, respectively, in animals (64). In contrast, both *Arabidopsis* and rice lack KDM6/JMJD3 and KDM2/JHDM1 group proteins (81); however, we expect that factors with H3K27 and H3K36 demethylation activities other than KDM6/JMJD3 and KDM2/JHDM1 group proteins exist in plants.

The biochemical properties and biological functions of histone demethylases are

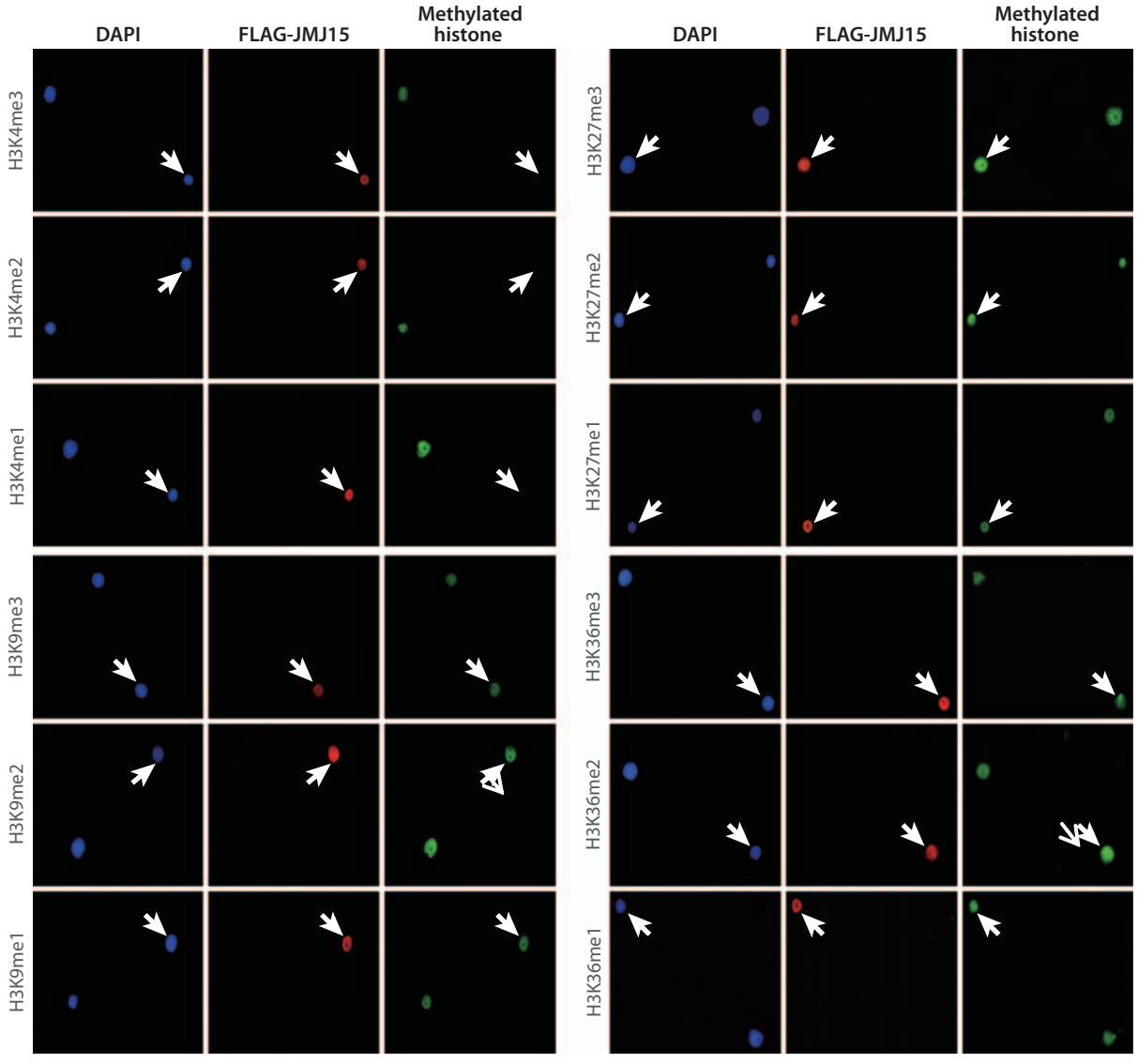


**Figure 5**

MEE27/JMJ15 is a histone H3K4 demethylase. MEE27/JMJ15 was transiently expressed in tobacco cells as a FLAG fusion protein and the nuclei were isolated for immunolabeling. DAPI (4,6-diamidino-2-phenylindole) staining (left panels) indicates the location of nuclei in each field. Indirect immunofluorescence with antibodies against FLAG (middle panels) or methylated histones (right panels) were used to analyze the substrate specificity of MEE27/JMJ15 in vivo. Cells exhibiting expression of MEE27/JMJ15 are marked by arrows. A complete loss of H3K4me3, H3K4me2, and H3K4me1 was observed in MEE27/JMJ15-expressing cells, but H3K9me3/2/1, H3K27me3/2/1, and H3K36me3/2/1 levels were not affected.

emerging. How these enzymes work, how these enzymes are recruited to their target loci, and their roles in development are still largely unknown. Further biochemical and genetic research would extend our knowledge about the dynamic control of histone methylation and how histone methylation programs plant development.

## SUMMARY POINTS

1. Several distinct lysine and arginine sites in histone tails are methylated by specific HKMTs or PRMTs to yield biological outputs in processes ranging from the regulation of genome stability to the execution of plant development.
2. The histone methylation modification is recognized by specific reader(s) that translate the epigenetic information into functional readouts.
3. The methylation marks can be erased by histone demethylases; therefore, histone methylation is dynamically regulated by writers and erasers.

## FUTURE ISSUES

1. It would be of great interest in the future to identify more writers as well as readers and study the mechanisms of writers targeting to specific loci and of effectors recognizing particular modifications to interpret the signal into specific biological functions.
2. Although several histone demethylase candidates have been characterized in plants, more biochemical and genetic evidence is needed to identify the intrinsic histone demethylases, including lysine and/or arginine demethylases, in plants and elucidate their roles in regulating plant development and genome stability.
3. Despite remarkable advances made in uncovering writers and erasers responsible for histone methylation, the mechanisms of recruiting histone methyltransferases and demethylases to their target sites are far from being understood. Locus specificity of histone methylation might be produced by recruiting different histone modifying enzymes by diverse sequence-specific transcription factors. Identification of proteins or protein complexes that interact directly with these enzymes will be one way to illuminate the mechanism by which histone modifications are written to specific loci.
4. The interplay among the great variety of epigenetic modifications is far from being fully understood. A comprehensive analysis of the plant epigenome is emerging, which will help to understand how these epigenetic modifications combine to regulate gene expression. Using materials specific to a single cell type or developmental stage might be helpful to achieve a better resolution.
5. How chromatin modifications program plant development and how these modifications communicate with endogenous and exogenous signals are topics of great interest for further research.
6. Another interesting avenue of research will be determining how methylation modification regulates other proteins in addition to histones. Although histones are by far the predominant substrates identified for HKMTs, PRMTs, and HDMs, a wide variety of methylated nonhistone proteins have been identified. For instance, G9a/KMT3C is found to methylate nonhistone proteins CDYL1, WIZ, ACINUS, and G9a (automethylation) (105). In addition, tumor suppressor and transcription factor p53 is modified by several different covalent modifications, including methylation, acetylation, and ubiquitination. Thus, it would not be surprising to find a wide range of other proteins that are

methylated on lysine or arginine residues. Identification of more such proteins and determination of how methylation regulates their function will improve our understanding of methyltransferases and demethylases.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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**Errata**

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