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REVIEW
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Writing and Reading Histone H3 Lysine 9 Methylation in *Arabidopsis*

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In eukaryotes, histone H3 lysine 9 methylation (H3K9me) mediates the silencing of invasive and repetitive sequences by preventing the expression of aberrant gene products and the activation of transposition. In *Arabidopsis*, while it is well known that dimethylation of histone H3 at lysine 9 (H3K9me2) is maintained through a feedback loop between H3K9me2 and DNA methylation, the details of the H3K9me2-dependent silencing pathway have not been fully elucidated. Recently, the regulation and the function of H3K9 methylation have been extensively characterized. In this review, we summarize work from the recent studies regarding the regulation of H3K9me2, emphasizing the process of deposition and reading and the biological significance of H3K9me2 in *Arabidopsis*.

Keywords: epigenetics, histone, heterochromatin, H3K9 methylation, transcriptional silencing

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INTRODUCTION

In eukaryotic cells, chromatin is divided into two major types of compartments: heterochromatin and euchromatin, reflecting the repressive and permissive potential for transcription in these regions, respectively (Ding et al., 2007). Chromatin is rich in repetitive sequences and transposable elements inside and near centromeres, posing a risk for genome instability through their potential for transposition and meiotic recombination. Thus, during the whole life cycle, it is necessary to keep these regions inaccessible, condensed, and transcriptionally silent. Such regions are classified as constitutive heterochromatin (Saksouk et al., 2015). In contrast, facultative heterochromatin refers to regions whose compaction and silencing are dynamic in the life cycle or under stress stimuli, mainly distributed in chromosomal arms (Trojer and Reinberg, 2007).

Chromatin states are modulated by modifications at the N-terminal tails of histones, DNA methylation, and different histone variants (Jenuwein and Allis, 2001). Histone H3K9 methylation is a critical marker for transcriptional silencing and heterochromatin formation, mostly constitutive heterochromatin formation. Methylation states at H3K9 can be mono- (H3K9me1), di- (H3K9me2), or tri- (H3K9me3) methylation. In mammals, H3K9me3 is the most abundant marker in constitutive heterochromatin (Peters et al., 2003; Rice et al., 2003). However, in plants, the modification of H3K9me1 and H3K9me2 is rich in constitutive heterochromatin and only slightly present in facultative chromatin, whereas H3K9me3 is distributed with a high concentration in euchromatin and at expressed genes (Naumann et al., 2005). In *Arabidopsis*, H3K9me3 methylation broadly marks 40% of all genes (Roudier et al., 2009), but only a low level of H3K9me3 can be detected in regions with transposons and pseudogenes (Charron et al., 2009). Thus, the function of H3K9me3 has been altered in *Arabidopsis* compared to H3K9me3 in yeast and mammals.

H3K9me2 is mainly catalyzed by the histone methyltransferases KRYPTONITE (KYP), SUVH5, and SUVH6 in *Arabidopsis* and is maintained through the feedback loop between H3K9me2 and non-CG methylation (Du et al., 2015). Several studies have shown more details of H3K9me2 deposition with the structural analysis of KYP/SUVH5/SUVH6 and their role in H3K9me2 deposition (Du et al., 2014; Li et al., 2018) and other H3K9 methyltransferases (Caro et al., 2012) and cofactors (Yu et al., 2017). The downstream part of H3K9me2-dependent silencing has also been investigated by identifying a novel H3K9 reader (Zhang C. et al., 2018; Zhao et al., 2019). In this article, we review the writing, reading, and biological roles of H3K9 methylation in *Arabidopsis*.

H3K9 METHYLTRANSFERASES IN *ARABIDOPSIS*

Histone lysine methyltransferases usually contain a catalytic SET domain, which is named after three *Drosophila melanogaster* genes, *Su(var)3-9*, *E(z)*, and *Trx* (Jenuwein et al., 1998). In fission yeast, there is only one H3K9 methyltransferase, Clr4/KMT1, which is responsible for all three states of H3K9 methylation (Nakayama et al., 2001; **Figure 1**). In mammals, there are multiple H3K9 methyltransferases with different catalytic activities and target genes (Sims et al., 2003; Dodge et al., 2004; Shinkai and Tachibana, 2011; **Figure 1**). SUV39H1 and SUVH39H2 mono- and dimethylase catalyze di-and trimethylation in constitutive heterochromatic regions, SETDB1 monomethylates at the pericentromeric region, and the heterodimer of G9a and G9a-like protein (GLP) catalyzes di- and trimethylation in euchromatic regions.

In *Arabidopsis*, there are 15 SET-domain proteins that are related to SU(VAR)3-9 (Baumbusch et al., 2001; Lei et al., 2012; Zhang and Ma, 2012; **Figure 1**). Ten of these proteins are classified as SU(VAR)3-9 HOMOLOGS (SUVH1-SUVH9), and the remaining five are classified as SU(VAR)3-9-RELATED proteins (SUVR1-SUVR5) (**Table 1**). Among the nine SUVHs, KYP/SUVH4, SUVH5, and SUVH6 have been well identified as H3K9 methyltransferases responsible for maintaining H3K9 methylation. KYP mediates the majority of H3K9me2 methylation in both constitutive and facultative heterochromatin in *Arabidopsis*, while SUVH5 and SUVH6 only play minor roles in H3K9me2 methylation (Jackson et al., 2002, 2004; Stroud et al., 2014; Li et al., 2018). Crystal structures of KYP, SUVH5, and SUVH6 reveal that the post-SET domain is critical for enzymatic activity (Li et al., 2018); thus, SUVH2 and SUVH9, which lack the post-SET domain, are enzymatically inactive (Johnson et al., 2014). The remaining SUVH1, SUVH3, SUVH7, and SUVH8 were recently reported to function in transcriptional activation but not silencing, expanding the roles of SUVHs in transcriptional regulation (Harris et al., 2018; Xiao et al., 2019). Nevertheless, SUVH7 and SUVH8 are both primarily expressed and imprinted in the endosperm (Gehring et al., 2011; Wolff et al., 2011), indicating an endosperm-specific targeting mechanism favoring a relatively specific chromatin environment. Indeed, SUVH7 has already been shown to play a role in establishing postzygotic hybridization barriers established by H3K9me2 (Wolff et al., 2015; Jiang et al., 2017). Interestingly, computational characterization predicts that SUVH7 and SUVH8 are capable of catalyzing H3K9me1 and H3K9me2 methylation. Two critical residues in the catalytic pocket, Tyr1124 and Phe1209, determine the product specificity in GLP, a G9a-related methyltransferase (Wu et al., 2010). Meanwhile, H3K9me1 or H3K9me2 is correlated with the presence of Tyr in one of the positions and non-Tyr in the other, indicating that the two SUVHs are capable of catalyzing H3K9me1 or H3K9me2. Thus, SUVH7 and SUVH8 may function as methyltransferases for endosperm-specific H3K9me2 deposition. Taken together, KYP, SUVH5, and SUVH6 are the general H3K9 methyltransferases in *Arabidopsis*, and it is possible that SUVH7 and SUVH8 act as endosperm-specific methyltransferases.

Among the five SUVRs, SUVR1, and SUVR2 have shown no HMTase activity in an *in vitro* enzymatic assay, but SUVR4 has HMTase activity to convert H3K9me1 to H3K9me2 (ubiquitin) and H3K9me3 (without ubiquitin) *in vitro* (Thorstensen et al., 2006; Veiseth et al., 2011). The level of H3K9me3 is correlated with the amount of SUVR4-GFP in *Arabidopsis* nuclei, but the effect of genome-wide H3K9mer3 has not been determined (Veiseth et al., 2011). SUVR5 is capable of establishing H3K9me2 in a DNA methylation–independent manner and is involved in the response to environmental or developmental cues (Caro et al., 2012).

TARGETING H3K9 METHYLATION THROUGH BINDING TO METHYLATED DNA

DNA methylation is tightly connected with H3K9 methylation. In *Neurospora crassa*, the H3K9 methyltransferase DIM5 establishes H3K9me3, and then heterochromatin protein 1 (HP1) recognizes H3K9me3 to facilitate the targeting of the DNA methyltransferase DIM2 (Tamaru and Selker, 2003). In mammals, knockout of either *G9a* or *Suv39 H1, Suv39 H2* results in reduced DNA methylation in mice (Ikegami et al., 2007). Moreover, H3K9 methylation is dependent on DNA methylation in human cancer cells (Espada et al., 2004). Likewise, in *Arabidopsis*, KYP, SUVH5, and SUVH6 are primarily recruited to the targets through SET and RING-associated (SRA) domain binding to DNA that is methylated in the CHG context (H stands for any base except G). H3K9me2 is known to recruit the DNA methyltransferases CMT2 and CMT3, which mediate CHH and CHG DNA methylation, respectively, in a feedback loop with H3K9me2 (**Figure 2**; Johnson et al., 2007; Bernatavichute et al., 2008; Du et al., 2012; Zemach et al., 2013; Stroud et al., 2014). Considering the targeting of H3K9 methyltransferases to CHG-methylated DNA, KYP, SUVH5, and SUVH6 have distinct DNA binding preferences. KYP, which is responsible for the majority of H3K9me2, has high affinity to the CWG (W stands for A or T) context but has low affinity to the CCG context (Li et al., 2018). The differential binding affinity is consistent with the phenotype that DNA methylation at CWG is strongly lost in *kyp*, but loss of CCG methylation is very low in *kyp* but high in *suvh5* and *suvh6*.

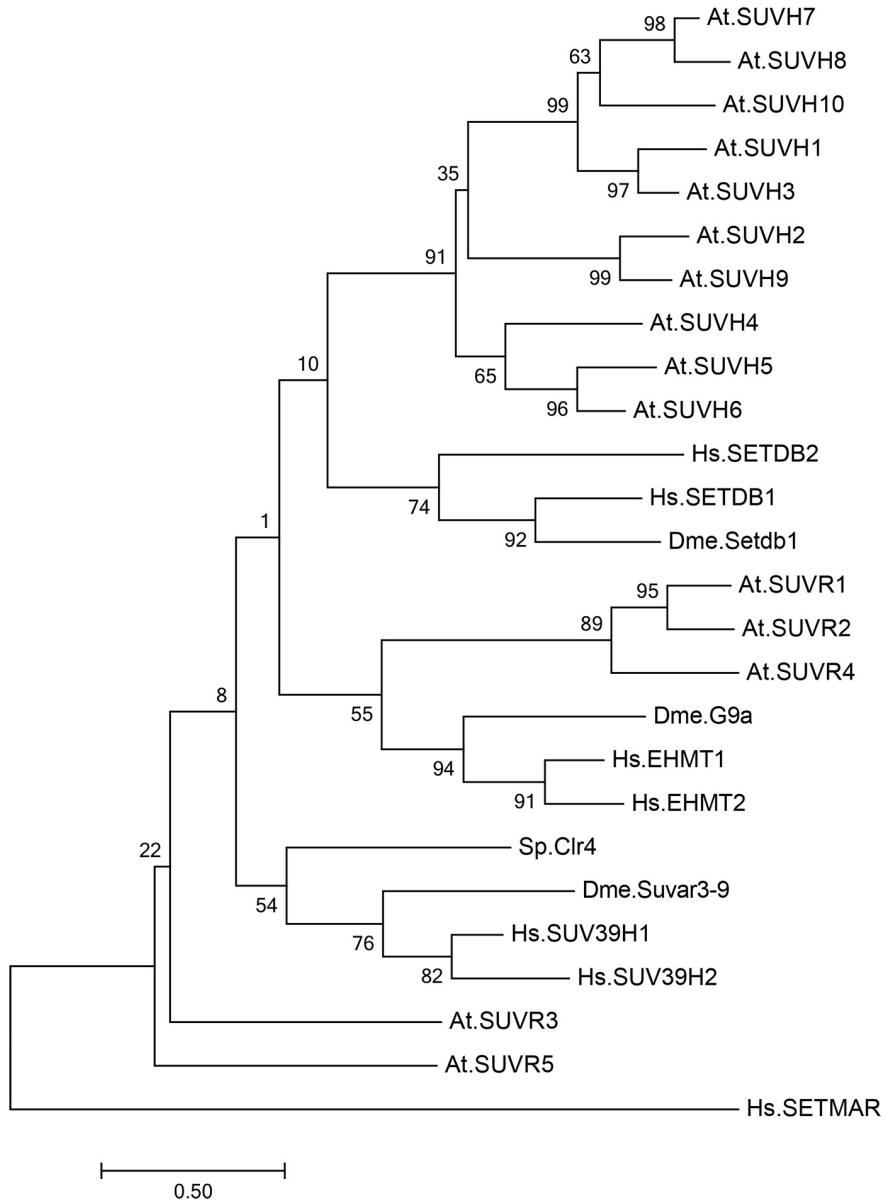

FIGURE 1 | Phylogenetic analysis of SU(VAR)3–9 homologous proteins in *Arabidopsis thaliana*, *Drosophila melanogaster*, *Schizosaccharomyces pombe*, and *Homo sapiens*. Phylogenetic analysis of 15 SU(VAR)3–9 homologous protein sequences from *Arabidopsis thaliana* (At), three SU(VAR)3–9 homologous protein sequences from *Drosophila melanogaster* (Dme), one SU(VAR)3–9 homologous protein sequence from *Schizosaccharomyces pombe* (Sp), and five SU(VAR)3–9 homologous protein sequences from *Homo sapiens* (Hs). The evolutionary history was inferred by using the maximum likelihood method based on the Poisson correction model. Phylogenetic analysis was performed using MEGA 7.0.

Consistent with the *in vivo* consequence of DNA methylation, SUVH5 has a preference for the CCG context, and SUVH6 can bind to both the CWG and CCG contexts, which act as a backup of KYP to ensure H3K9me2 in all CHG contexts (**Figure 2**).

While the feedback loop between CHG DNA methylation is frequently discussed, CG and CHH methylation also contribute to H3K9me2 deposition through SRA domains of KYP, SUVH5, and SUVH6 binding to DNA that is methylated in CG or CHH context. In a large-scale comparative epigenome analysis, *MET1* was indeed found to be required for the maintenance of CMT2-dependent asymmetric CHH methylation at loci with H3K9me2 (Zhang Y. et al., 2018). Moreover, SUVH5 and SUVH6 can bind to DNA that is methylated in the CG context *in vitro* (Li et al., 2018), supporting the view that CG methylation also contributes to H3K9me2 deposition. In addition to CG methylation, it has been known for many years that CHH methylation generated by the RNA-directed DNA methylation (RdDM) pathway is also involved in H3K9me2 deposition (Wierzbicki et al., 2008; Zheng et al., 2009; Shin et al., 2013; Liu Z.W. et al., 2014). Recent biochemical

TABLE 1 | Summary of DNA methyltransferases and SUV methyltransferases in *Arabidopsis thaliana.*

Gene ID	Gene name	Description
AT5G49160	MET1	Maintains CG methylation (Finnegan et al., 1996)
AT4G19020	CMT2	Deposits mainly CHH methylation (Stroud et al., 2014)
AT1G69770	CMT3	Maintains CHG methylation (Lindroth et al., 2001)
AT5G14620	DRM2	Establishes *de novo* CHH methylation (Cao and Jacobsen, 2002)
AT5G04940	SUVH1	Required for transcriptional activation (Harris et al., 2018)
AT2G33290	SUVH2	Recruit RNA polymerase V to establish CHH methylation (Johnson et al., 2008; Johnson et al., 2014)
AT1G73100	SUVH3	Required for transcriptional activation (Harris et al., 2018)
AT5G13960	SUVH4	Maintains H3K9me1/me2 (Jackson et al., 2002)
AT2G35160	SUVH5	Maintains H3K9me1/me2 (Ebbs and Bender, 2006)
AT2G22740	SUVH6	Maintains H3K9me1/me2 (Jackson et al., 2004)
AT1G17770	SUVH7	Paternal-expressed imprinted gene (Gehring et al., 2011; Wolff et al., 2011)
AT2G24740	SUVH8	Maternal-expressed imprinted gene (Gehring et al., 2011; Wolff et al., 2011)
AT4G13460	SUVH9	Recruits RNA polymerase V to establish CHH methylation (Johnson et al., 2008; Johnson et al., 2014)
AT2G05900	SUVH10	Pseudogene (Baumbusch et al., 2001)
AT1G04050	SUVR1	Unknown
AT5G43990	SUVR2	deposits H3K9me1/me2; H4K20me; H3K27me2 (Han et al., 2014)
AT3G03750	SUVR3	Unknown
AT3G04380	SUVR4	Deposits H3K9me2/me3 (Thorstensen et al., 2006; Veiseth et al., 2011)
AT2G23740	SUVR5	Establishes H3K9me2 independently of DNA methylation (Caro et al., 2012)

evidence indeed supports this hypothesis; all three SUVHs can bind to CHH-methylated DNA (**Figure 2**), and there is no sequence preference among the three SUVHs in targeting CHH-methylated DNA (Li et al., 2018).

Apart from the specificity of the SRA domain on the sequence context, other factors may also affect KYP, SUVH5, and SUVH6 targeting to methylated DNA. It was reported that SUVH4 and SUVH5 prefer to control transposable elements, but SUVH4 and SUVH6 prefer to target transcribed inverted repeat sources of dsRNA. Thus, in addition to DNA methylation states, chromatin state may also govern SUVH activities (Ebbs and Bender, 2006).

TARGETING H3K9 METHYLATION INDEPENDENTLY OF DNA METHYLATION

Apart from DNA methylation-dependent H3K9me2 deposition, there are known exceptions. G9a is one of the primary enzymes for H3K9me1 and H3K9me2 and usually interacts with another enzyme, GLP, to form a heteromeric complex that appears to be a functional H3K9 methyltransferase *in vivo* (Shinkai and Tachibana, 2011). In murine embryonic stem cells (mESCs), H3K9me2 at the newly integrated proviral LTR is reduced in cells with G9a silencing. Since there is no H3K9me2 or DNA methylation at the newly integrated region, *G9a* is considered to be responsible for *de novo* H3K9me2 (Leung et al., 2011). In addition to mESCs, G9a-dependent H3K9me2 has also been associated with gene repression in multiple human cell lines (Chen et al., 2009; Liu C. et al., 2014; Yoshida et al., 2015; Kramer, 2016; Scheer and Zaph, 2017).

Similar exceptions also exist in *Arabidopsis*. It was reported that SUVR5 is able to establish H3K9me2 independently of DNA methylation (**Figure 2**; Caro et al., 2012). Unlike KYP/SUVH5/SUVH6, SUVR5 does not have the SRA domain which can bind at methylated DNA but relies on a set of three C2H2 zinc fingers in tandem, which can bind at the sequence context of "TACTAGTA" *in vitro*. This motif also occurs at a minor part of transposable elements (TEs) and surrounds substantial genes losing H3K9me2 in *suvr5*, further supporting the role of zinc fingers in targeting H3K9me2 deposition. While H3K9 methyltransferases in yeast or mammals do not contain zinc fingers, DNA binding proteins recruiting H3K9 methyltransferases contain zinc fingers (Kim and Huang, 2003; Fog et al., 2012; Bian et al., 2015). In mammalian cells, ZNF644 has eight zinc finger motifs and WIZ contains 12 zinc finger motifs that are the binding partners of the G9a-GLP complex (Bian et al., 2015). The N-terminus of ZNF644 interacts with the transcriptional domain (TAD) of G9a, but the C-terminus of WIZ interacts with the TAD of GLP to facilitate the targeting of the G9a-GLP complex at specific genomic loci with the preference of the promoter region (Bian et al., 2015). Thus, it seems that targeting H3K9me2 by the zinc finger domain is a conserved mechanism in plants and mammals, but in plants, the zinc finger domain has been integrated into H3K9 methyltransferase. Interestingly, the combination of zinc fingers and a C-terminal SET domain can be found in all plant species (Caro et al., 2012). Thus, SUVR5 depositing H3K9me2 independently of DNA methylation might be conserved in plants. Another exception in *Arabidopsis* is SUVR4, which can bind to

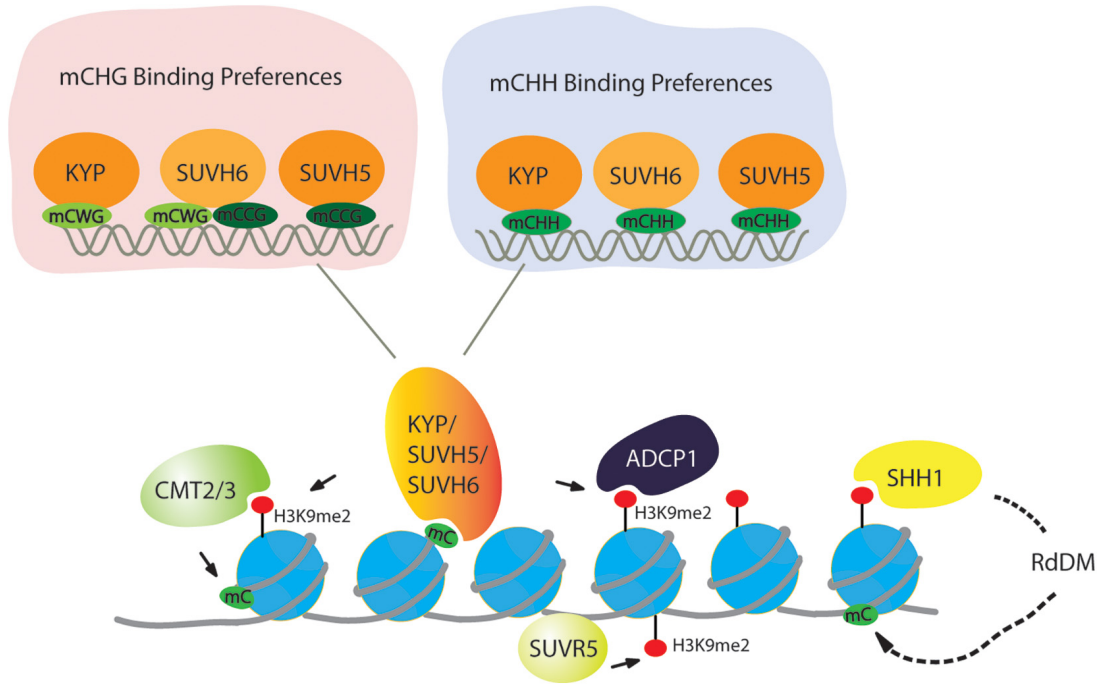

FIGURE 2 | Targeting of H3K9 dimethylation in a DNA methylation-dependent and -independent manner. For DNA methylation, CMT2 and CMT3 recognize the H3K9me2 mark and catalyze DNA methylation in the CHG and CHH context (H = A, T, or C), respectively. For H3K9 methylation, histone methyltransferases KYP/SUVH4, SUVH5, and SUVH6 bind at methylated DNA in the CHG and CHH context to deposit H3K9me2, creating a reinforcing loop between DNA methylation and Histone modification. In the CHG context, KYP has a preference of mCWG (W = A or T), while SUVH5 and SUVH6 have a high affinity to the mCCG. All three SUVHs have similar sequence specificities at mCHH sites. SUVR5 binds to DNA through its zinc finger domain to facilitate H3K9me2 independently of DNA methylation. H3K9me2 is captured by SHH1, then through RdDM pathway to methylate DNA.

ubiquitin through the N-terminal WIYLD domain to facilitate the conversion from H3K9me1 to H3K9me3 *in vitro*, but it is not clear if the WIYLD domain binds to ubiquitin *in vivo* and if this domain binds to histone or other proteins with ubiquitination (Thorstensen et al., 2006; Veiseth et al., 2011). Recently, it was reported that the CRL4DCAF8 ubiquitin ligase is capable of targeting H3 for polyubiquitination at K79 in mice, which may further promote H3K9me2 deposition (Li et al., 2017), suggesting a similar connection between histone ubiquitination and H3K9me3. Taken together, the deposition of H3K9me2 is not only DNA methylation dependent but can also be independent.

OTHER PROTEINS PARTICIPATE IN H3K9 METHYLATION DEPOSITION

The distribution of histone acetylation is usually anti-correlated with histone methylation, such as H3K9Ac and H3K9me2 (Zhou et al., 2010), indicating that the removal of H3K9Ac or relevant protein complexes functions in H3K9me2 deposition. Histone deacetylation is processed by histone deacetylases (HDACs), which play important roles in chromatin regulation (Liu X. et al., 2014). In mammals, SUV39H1 can interact with HDAC1 and HDAC2 (Vaute et al., 2002). Moreover, transcriptional repression by SUV39H1 is abolished by treatment with the HDAC inhibitor trichostatin A (TSA), indicating that the function of SUV39H1 is dependent on HDAC activity (Vaute et al., 2002). Likewise, in *Arabidopsis*, one of the HDACs, HDA6, also physically interacts with H3K9 methyltransferases KYP, SUVH5, and SUVH6, regulating a group of transposable elements and repetitive sequences (Yu et al., 2017). The mutant with compromised HDA6 has reduced H3K9me2 levels compared with the wild type, suggesting that H3K9me2 deposited by KYP, SUVH5, and SUVH6 is partly dependent on HDA6, but it is not clear that H3K9me2 deposition in *Arabidopsis* is dependent on the level of H3K9Ac at the targets or depends on the interaction between HDA6 and KYP/SUVH5/SUVH6, or perhaps both mechanisms exist in *Arabidopsis*, which has not been clearly dissected to date. Given the remaining H3K9me2 level in the *hda6* mutant, it will be interesting to know if other HDACs are also involved in H3K9me2 deposition in the future.

Matrix attachment regions (MARs) are important for chromatin organization and gene expression (Tetko et al., 2006; Zhao et al., 2014). MARs are stretches of AT-rich sequences that guide the binding of DNA to the nuclear matrix by recruiting MAR-binding proteins. Proteins with AT-hook motifs bind to MARs and play roles in regulating H3K9me2 levels. In *Neurospora crassa*, CHAP, a protein with AT-hook motifs, was demonstrated to recognize heterochromatic regions through AT-hook motifs and to recruit the H3K9 methyltransferase DIM5

to targets (Honda et al., 2016). In *Arabidopsis*, overexpression of AT-hook motif nuclear localized 22 (AHL22) causes delayed flowering time by increasing H3K9me2 at MAR located in an intron of the FLOWERING LOCUS T (FT) locus (Xiao et al., 2009; Yun et al., 2012). AHL16 regulates the expression of the floral repressor genes FLOWERING LOCUS C (FLC) and FLOWERING WAGENINGEN (FWA) by adjusting the H3K9me2 level (Xu et al., 2013). Overexpressed AHL10 increases genome-wide H3K9me2 levels in the endosperm of triploid seeds (Jiang et al., 2017). Consistent with the distribution of MARs that are mainly in chromosome arms, TEs that are methylated by H3K9me2 *via* AHL10 in the endosperm of triploid seeds are usually euchromatic AT-rich TEs (Jiang et al., 2017). To date, it has not been determined how AHLs regulate H3K9me2 levels. While there is no direct physical interaction between AHLs and H3K9 methyltransferase in *Arabidopsis*, AHLs usually interact with HDAC complexes both *in vitro* and *in vivo*, such as AHL22 interacting with HDA1, HDA6, and HDA9 (Xiao et al., 2009) and AHL16 interacting with FVE and MSI5, which are core components of the HDA6 complex (Gu et al., 2011; Xu et al., 2013). Thus, it is possible that AHLs participate in H3K9me2 deposition through interaction with HDACs, especially HDA6. Nevertheless, other chromatin-relevant proteins also occur in AHL complexes, such as SUVH9 in the AHL10 complex. Apart from interacting with the DDR complex and mediating Pol V recruitment in RdDM (Johnson et al., 2014; Liu Z.W. et al., 2014), SUVH9 also interacts with MORC6 and its two close homologs, MORC1 and MORC2, required for heterochromatin condensation and formation of 3D chromatin architecture at SUPPRESSOR OF DRM1 DRM2 CMT3 (SDC) and Solo-LTR loci (Jing et al., 2016). Recently, the mammalian nuclear matrix protein scaffold attachment factor B (SAFB) was found to participate in stabilizing heterochromatin architecture partially through phase separation, which is a phenomenon in which different biological molecules spontaneously separate into two coexisting liquid phases and result in miscellaneous non-membrane-bound cellular compartments. Depletion of SAFB results in more interchromosomal interactions around pericentromeric heterochromatin and a decrease in genomic compartmentalization, which could result from the decondensation of pericentromeric heterochromatin (Huo et al., 2020). Thus, it is also possible that AHLs and MARs participate in H3K9me2 regulation by affecting heterochromatin architecture and phase separation.

H3K9 READERS IN *ARABIDOPSIS*

H3K9 methylation recruits downstream effectors containing specific reader domains to further mediate gene silencing. In metazoans, heterochromatin protein 1 (HP1) is known to read the trimethylated lysine 9 residue of histone H3 (H3K9me3) (Bannister et al., 2001; Jacobs et al., 2001), which is a hallmark histone modification for transcriptionally silenced heterochromatin in mammals (Zeng et al., 2010). HP1 contains a conserved chromodomain (CD) at the N-terminus and a chromo shadow domain (CSD) at the C-terminus (Li et al., 2002). CD is able to directly bind to H3K9me3 (Jacobs et al., 2001). Based on sequence similarity and early biochemistry analyses, the homolog of HP1 in *Arabidopsis*, LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) was first proposed to be the H3K9me reader that mediates H3K9me2-dependent heterochromatic silencing, as it was shown to bind H3K9me2 *in vitro* (Jackson et al., 2002). Nevertheless, several lines of evidence have indicated that LHP1 is a plant-specific PRC1 H3K27me3 reader subunit. The CHROMO domain of LHP1 specifically binds to H3K27me3 but not H3K9me in *Arabidopsis,* and the genome-wide distribution of LHP1 displays significant overlap with H3K27me3-enriched sites (Turck et al., 2007; Exner et al., 2009; Lu et al., 2011). While SHH1, CMT2, and CMT3 have the capability to bind to histones with H3K9me2 (Law et al., 2013; Stroud et al., 2014; **Figure 1**), their function is to maintain or initiate non-CG methylation but not the downstream H3K9me2 reader; therefore, the reader of H3K9me2 in plants had not been determined until two recent studies were conducted in *Arabidopsis*. Agenet domain (AGD)-containing p1 (AGDP1), also known as ADCP1, appears to be a plant-specific H3K9 reader and functions as an HP1 equivalent protein (Zhang C. et al., 2018; Zhao et al., 2019). The tandem AGDs of AGDP1 can specifically recognize H3K9me2 and unmethylated K4 on the H3 tail (H3K4me0) through two negatively charged surface pockets. In structural studies, AGD12 adopts a tandem Tudor-like conformation, which resembles the human UHRF1 tandem Tudor and *Arabidopsis* SHH1 SAWADEE domains, both of which function as H3K9me2 readers with similar recognition mechanisms (Arita et al., 2012; Cheng et al., 2013; Law et al., 2013). ADCP1 is responsible for H3K9me2-dependent silencing, and the *in vivo* binding site of ADCP1 largely overlaps with the regions enriched by H3K9me2, further supporting that ADCP1 is indeed an H3K9me2 reader (Zhao et al., 2019).

While ADCP1 has been successfully identified, how ADCP1 mediates H3K9me2-dependent transcriptional silencing still needs to be discovered. Given that ADCP1 is essential for heterochromatin formation and TE silencing, but ADCP1 itself is only a histone binding protein without any repressor domain (Zhao et al., 2019), other chromatin modeling proteins must be recruited by ADCP1 to heterochromatin. Recently, SMC4, a core subunit of condensins I and II, was identified to act in conjunction with CG methylation, CHG methylation, the chromatin remodeler DDM1 (DECREASE IN DNA METHYLATION 1), and histone modifications, including H3K9me2 and H3K27me1 (Wang et al., 2017). Considering the function of SMC4 in H3K9me2-mediated transcriptional silencing (Wang et al., 2017), it is worth knowing whether SMC4 works together with ADCP1 to mediate heterochromatic silencing. Another possibility is that ADCP1 mediates downstream silencing by driving nucleosome phase separation. It has been demonstrated that human HP1α and *Drosophila* HP1a may demix from aqueous solution to form phase-separated droplets (Larson et al., 2017; Strom et al., 2017), which rapidly induce compacted chromatin. Similarly, ADCP1 can mediate heterochromatin phase separation together

with reconstituted nucleosomes bearing H3K9me3 *in vitro* (Zhao et al., 2019). Thus, ADCP1 probably has a similar ability to mediate phase separation as the functional analog of mammalian HP1.

ROLE OF H3K9 METHYLATION IN *ARABIDOPSIS* DEVELOPMENT

The mutant with compromised KYP, SUVH5, and SUVH6 has no obvious abnormality in development; thus, H3K9 was considered to play minor roles in *Arabidopsis* development. Nevertheless, the role of H3K9me2 in *Arabidopsis* development has been identified with more careful observations and new approaches.

The main H3K9me2 methyltransferase, KYP, was proven to repress primary seed dormancy by suppressing the expression of dormancy and ABA pathway-related genes, such as *DOG1*, which is a master regulator in the control of seed dormancy (Bentsink et al., 2006), and *ABI3* and *ABI4,* which are components of ABA signaling (Koornneef et al., 2002; Zheng et al., 2012). However, evidence that H3K9me2 directly regulates the expression of these genes *via* H3K9me2 levels is not available. Until recently, SUVH5 was revealed to directly repress the expression of genes related to the ABA signaling pathway, *DOG*1, and its homologs *via* H3K9me2 in light-mediated seed germination (Gu et al., 2019). Thus, SUVH5-mediated H3K9me2 directly participates in controlling seed germination in *Arabidopsis*. After seed germination, plants enter the vegetative stage. While the role of H3K9me2 in the vegetative stage is not clear, H3K9me2 is crucial for the transition to flowering. Knockdown of *AHL16* leads to obvious late flowering, which results in increased expression of two flowering repressors, *FLOWERING LOCUS C* (*FLC*) and FWA. Consistent with the increased expression pattern, the H3K9me2 levels at the intron of *FLC* and *FWA* loci were reduced. Interestingly, the phenotype of late flowering in the *ahl16* mutant only occurs in Landsberg (Ler) accession but not in Columbia (Col) accession, indicating the ecotype-dependent regulation process (Xu et al., 2013).

During male meiosis, H3K9me2 is crucial for the distribution of meiotic recombination (Underwood et al., 2018). In plants, meiotic recombination is enriched in euchromatic regions, rather than pericentromeric heterochromatin, associated with H3K4me3 and histone variant H2A.Z but inversely correlated with DNA methylation. Suppression of meiotic recombination within the centromeric region is thought to be important for maintaining the fidelity of genome transmission during meiosis (Choi et al., 2018). Loss of DNA methylation in the *met1* mutant leads to epigenetic activation of meiotic double-strand breaks (DSBs) in proximity to centromeres (Choi et al., 2018). In addition, non-CG methylation and the H3K9me2 pathway are also responsible for suppressing pericentromeric recombination (Underwood et al., 2018). Epigenetic activation of recombination and crossovers (COs) can be induced *via* loss of H3K9me2 and non-CG methylation in the *kyp*, *suvh5*, *suvh6*, or *cmt3* mutant (Underwood et al., 2018), making it possible to induce COs near centromeres, which are otherwise very low-frequency CO regions in *Arabidopsis* and crops (Taagen et al., 2020).

In *Arabidopsis thaliana* and *Arabidopsis lyrata* seed development, H3K9me2 and CHG methylation are involved in the regulation of genomic imprinting that leads to differential expression of parent-of-origin alleles by maintaining or reinforcing the repression of maternal alleles of imprinted paternally expressed genes (PEGs) (Klosinska et al., 2016; Moreno-Romero et al., 2019). Moreover, the presence of the three repressive epigenetic marks H3K27me3, H3K9me2, and CHG methylation on the maternal alleles in endosperm can be considered a specific epigenetic signature of paternally expressed imprinted genes in the endosperm of *Arabidopsis* (Moreno-Romero et al., 2019). These marks are able to predict known PEGs at high accuracy and identify several new PEGs that were confirmed by INTACT-based endosperm transcriptomes (Moreno-Romero et al., 2019). In addition to maintaining genomic imprinting in the endosperm of diploid *Arabidopsis* seeds, H3K9me2 also functions in establishing a hybridization barrier from interploidy cross in the endosperm of triploid seeds (the triploid block) (Jiang et al., 2017). The triploid block acts as an instant reproductive barrier that prevents backcrossing of the newly formed polyploid plants with their progenitors (Schatlowski and Kohler, 2012). Multiple PEGs are enhanced in the endosperm of triploid seeds (Kradolfer et al., 2013; Wolff et al., 2015). Increased H3K9me2 levels in AT-rich TEs derived from overexpressed ADM and AHL10 contribute to enhancing the expression of PEGs, such as PEG2, which is a crucial component in establishing the triploid block. Moreover, H3K9me2 levels in AT-rich TEs are also associated with the different phenotypes of the triploid block in Col and Ler accessions.

CONCLUSION AND PERSPECTIVE

In plants, H3K9 methylation, mainly H3K9me2, functions importantly in suppressing TEs and repetitive sequences, protecting plant genomes from TE transposition and genome instability. To enable plants to correctly deposit H3K9me2 in the genome, multiple H3K9 methyltransferases are in charge of H3K9me2 deposition in different sequence contexts *via* DNA methylation-dependent and -independent activities. Apart from playing a role in genome stability, H3K9me2 also plays roles in plant development and environmental stimuli. Recent studies have enhanced our understanding of the structure and recruitment of H3K9 methyltransferases and the downstream effector of H3K9me2, but open questions remain.

Given that H3K9me2 plays important roles in plant development and environmental stress, how the H3K9 methylation pathway is in response to developmental cues or environmental stimuli will be highly interesting to explore. In addition, our mechanistic understanding of downstream effectors of H3K9 methylation is also limited, while the H3K9me reader has been identified in *Arabidopsis*. The mechanism by which ADCP1 mediates transcriptional silencing, the existence of other H3K9me downstream effectors, the role of phase separation

in chromatin condensation *in vivo*, and how H3K9me functions in response to developmental cues or environmental stimuli remains to be elucidated. Answering these questions will further broaden our understanding of H3K9 methylation-dependent transcriptional silencing.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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