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Arabidopsis Histone Lysine Methyltransferases

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Abstract

In eukaryotes, changes in chromatin structure regulate the access of gene regulatory sequences to the transcriptional machinery and play important roles in the repression of transposable elements, thereby protecting genome integrity. Chromatin dynamics and gene expression states are highly correlated, with DNA methylation and histone post-translational modifications playing important roles in the establishment or maintenance of chromatin states in plants. Histones can be covalently modified in a variety of ways, thereby affecting nucleosome spacing and/or higher-order nucleosome interactions directly or via the recruitment of histone-binding proteins. An extremely important group of chromatin modifying enzymes are the histone lysine methyltransferases (HKMTs). These enzymes are involved in the establishment and/or maintenance of euchromatic or heterochromatic states of active or transcriptionally repressed sequences, respectively. The vast majority of HKMTs possess a SET domain named for the three Drosophila proteins that are the founding members of the family: *S*uppressor of variegation, *E*nhancer of zeste and *T*rithorax. It is the SET domain that is responsible for *HKMT* enzymatic activity. Mutation of Arabidopsis HKMT genes can result in phenotypic abnormalities due to the improper regulation of important developmental genes. Here, we review the different classes of HKMTs present in the model plant *Arabidopsis thaliana* and discuss what is known about their biochemical and biological functions.

I. INTRODUCTION

In eukaryotes, nuclear DNA is organized by histone proteins to form the fundamental unit of chromatin, the nucleosome. Each nucleosome is composed of 147 base pairs of DNA that is wrapped not quite twice around a histone octamer composed of two copies each of histone H2A, H2B, H3 and H4 (Luger et al., 1997). It is now clear that chromatin assembly exerts a major influence on gene expression by affecting the accessibility of the transcriptional machinery, including RNA polymerase complexes and transcription factors, to the DNA. As a consequence, changes in chromatin structure accompany a broad spectrum of important processes during development, including differentiation, embryonic stem cell maintenance and senescence (Baroux et al., 2007; He and Amasino, 2005; Hochedlinger and Plath, 2009; Kouzarides, 2007).

Nucleosome positioning is highly dependent on the genome sequence itself (Kaplan et al., 2009; Segal et al., 2006). The accessibility of DNA sequences within each nucleosome is further modulated by covalent modifications of the histones, methylation of cytosines in the DNA that is wrapped around the histones and differential use of histone variants. In vertebrates and plants, post-translational modification of histones and DNA methylation regulate or reflect the chromatin condensation and transcriptional status of the associated DNA. Genes located in a condensed chromatin context (heterochromatin) are generally

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inactive or silenced, whereas those found in a decondensed chromatin context (euchromatin) are more likely to be transcribed (Jenuwein and Allis, 2001; Kouzarides, 2007).

Heterochromatin is typically enriched in repetitive DNA, including transposable elements, centromeric repeats and excess, inactive ribosomal RNA (rRNA) gene repeats. Unlike constitutive heterochromatin, which remains condensed throughout the cell cycle, euchromatic regions undergo dynamic changes in chromatin condensation state and include intervals, such as intergenic sequences, that are often characterized by the presence heterochromatic marks (Bender, 2004a).

Changes in DNA methylation or histone modification states are mediated by specific enzymes. With regard to histone post-translational modifications, the enzymes modifying histones H3 and H4, particularly within their N-termini that protrude from the nucleosome core, are best understood (Kouzarides, 2007). The modifications carried out by these enzymes include methylation, acetylation, phosphorylation, ubiquitination, sumoylation, citrullination and ADP-ribosylation. The large variety of histone modifications, potentially conferring regulatory information, have been hypothesized to constitute a so-called ‘histone code’ (Jenuwein and Allis, 2001; Kouzarides, 2007).

Histone methylation occurs at both lysine and arginine amino acids and is used to mark both active and inactive chromatin, depending on context (Lachner and Jenuwein, 2002; Wang et al., 2007; Yu et al., 2006). For instance, Histone 3 Lysine 4 (H3K4) that is mono-, di- or trimethylated is present in nucleosomes associated with the promoter regions of active genes, whereas Histone 3 Lysine 9 (H3K9) mono-, di- and trimethylation occurs in nucleosomes associated with inactive genes located in euchromatic region and within highly condensed constitutive heterochromatin (Bernatavichute et al., 2008; Gendrel et al., 2002; Zhang et al., 2009).

Repressive histone modifications and DNA methylation are mechanistically linked (Richards, 2002). For example, mutations in the cytosine methyltransferase, MET1, lead to decreased H3K9 dimethylation, whereas mutations disrupting the functions of the H3K9 methyltransferase, Kryponite/SUVH4 (SU(VAR)3–9 homologues 4) results in decreased cytosine methylation (Jackson et al., 2002, 2004; Tariq et al., 2003). Genome-wide analyses have also revealed correlations between patterns of histone modification and cytosine methylation (Bernatavichute et al., 2008; Cokus et al., 2008; Gendrel et al., 2002; Lister et al., 2008; Zhang et al., 2009).

In Arabidopsis, a family of genes encode putative histone methyltransferases. Some of these enzymes function as arginine methyltransferases, but the majority are believed to be histone lysine methyltransferases (HKMTs) (Baumbusch et al., 2001; Ng et al., 2007; Niu et al., 2007; Wang et al., 2007). Five lysine methylation sites have been identified so far in plants, namely lysines 4, 9, 27 and 36 of Histone 3 and lysine 20 of Histone 4 (Pfluger and Wagner, 2007; Zhang et al., 2007b). In other eukaryotes, methylation of H3K79, H4K59 and H1BK26 has also been reported (Trojer et al., 2007; Zhang et al., 2003). All known HKMTs in plants have a so-called SET (Suppressor of variegation, *E*nhancer of zeste and *T*rithorax) domain that is responsible for the catalytic activity of the enzymes. Thus, these proteins are members of the SET Domain Group (SDG) protein super-family (Gendler et al., 2008). Our review focuses on those classes of SDG proteins that are known, or thought, to possess HKMT activity.

II. HKMT CLASSIFICATION IN ARABIDOPSIS

A. GENE ORGANIZATION AND EVOLUTION

In *Arabidopsis thaliana*, 49 genes encoding putative SET domain-containing proteins have been identified (www.chromDB.org; Baumbusch et al., 2001; Ng et al., 2007). Similarly, the human genome encodes 50 SDG proteins, including 24 HKMTs. By contrast, the budding yeast (*Saccharomyces cerevisiae*) genome encodes only four SDG proteins (Fig. 1) (Allis et al., 2007). Of the 49 *A. thaliana* SDG proteins, 31 are known, or thought, to have HKMT activity and can be divided into five classes (I to V), based on their domain architectures (Fig. 2) and/or differences in enzymatic activity (Fig. 3) (Baumbusch et al., 2001; Ng et al., 2007; Springer et al., 2003). A class VI, which contain SDG proteins with a disrupted SET domain, and a class VII, which include SDG proteins that methylate non-histone proteins, have also been described but are not discussed in this review (Ng et al., 2007).

Phylogenetic analyses of *A. thaliana* and *Zea mays* genes have indicated that most of the gene duplication and functional diversification events that gave rise to the SDG protein family occurred prior to the divergence of monocotyledonous and dicotyledonous plants (Ng et al., 2007; Springer et al., 2003). However, there are exceptions, as exemplified by class III SDG genes that encode *T*rithora*x*-like (ATX) proteins. The Arabidopsis genome encodes five ATX genes, whereas rice and maize have only two or three, respectively (Ng et al., 2007; Springer et al., 2003). This observation suggests that duplication and diversification of some ATX genes occurred after the divergence of monocots and dicots, resulting in two sub-groups: sub-group 1 including ATX1 and ATX2 in Arabidopsis and two ATX2-like in maize and subgroup 2 including ATX3, ATX4 and ATX5 in Arabidopsis and a single copy of ATX4-like in maize (Fig. 1) (Avramova, 2009; Ng et al., 2007).

B. THE SET DOMAIN OF HKMT ENZYMES

Lysines can be mono-, di- or trimethylated, with differences in methylation state impacting or reflecting chromatin structure and gene transcriptional activity (Lachner and Jenuwein, 2002; Pfluger and Wagner, 2007). All known lysine methylation modifications, with the exception of Histone 3 Lysine 79 methylation (which has not been reported in plants), are carried out by methyltransferases that contain a SET domain. The SET domain encompasses approximately 130–150 amino acids that is thought to have evolved from an ancient motif found in bacterial proteins (Alvarez-Venegas et al., 2007). Structural and functional analyses of the SET domain of the human protein SUV39H1, which methylates H3K9, identified a series of key amino acids that are conserved in all SET-domain group proteins (Rea et al., 2000). Crystal structures of SET domains of several HKMTs have been solved, providing insights into their catalytic mechanisms and protein substrate specificities (Couture et al., 2005, 2006a, b; Xiao et al., 2003a, 2005). The SET domain possesses a unique fold dominated by 12 β-strands (Couture and Trievel, 2006). Two others domains, the pre-SET and the post-SET domains, sometimes flank the SET domain and may facilitate interactions with specific histone substrates. The hydroxyl group of a highly conserved tyrosine in the SET domain interacts with the substrate and transfers a methyl group to the lysine using *S*-adenosylmethionine (AdoMet) as the methyl group donor (Couture and Trievel, 2006; Rea et al., 2000; Xiao et al., 2003b).

III. CLASS I—IV HKMTS AND THEIR ROLES IN PLANT DEVELOPMENT

A. CLASS I HKMT ENZYMES

Class I HKMTs are homologues of *E*nhancer of *Z*este E(Z) from Drosophila that have H3K27 methyltransferase activity (Jones and Gelbart, 1993; Muller et al., 2002). The Arabidopsis genome encodes three E(Z)-like proteins: *C*URLY *L*EA*F* (CLF), *ME*DEA

(MEA) and *SWI*NGER (SWN), each of which contain a SET domain, two E(Z) domains, a SANT (SWI3, ADA2, N-CoR and TFIIIB DNA-binding) domain and a CXC (cysteine-rich) region. E(Z)-like proteins are components of Arabidopsis *P*olycomb *R*epressive *C*omplex 2 (PRC2)-like complexes that function astranscriptional repressors in diverse eukaryotes (Baroux et al., 2007). Analysis of *clf*, *mea* and *swn* mutants suggests that PRC2 complexes involving these proteins are required for H3K27 trimethylation, but direct biochemical evidence is currently lacking (Fig. 3) (Gehring et al., 2006; Makarevich et al., 2006).

CLF and *MEA* were the first HKMT genes described in plants, and helped underscore the importance of chromatin modification for proper plant development. CLF is required to repress *FLOWERING LOCUS C* (*FLC*) (Jiang et al., 2008; Wood et al., 2006). *FLC*, in turn, is a repressor of flowering, which is a process requiring a number of chromatin modifications, including histone methylation (He and Amasino, 2005). CLF is required for the methylation of H3K27 among histones associated with the FLC gene, as well as other developmentally important genes. Thus, *clf* mutations induce pleiotropic phenotypic defects in addition to altered flowering time, including altered leaf shape – hence the gene name (Katz et al., 2004; Makarevich et al., 2006; Schubert et al., 2006).

MEA is necessary for proper seed development. Maternally inherited loss of function *mea* alleles cause embryo abortion and endosperm over-proliferation (Grossniklaus et al., 1998; Kiyosue et al., 1999). Phylogenetic and molecular analyses have shown that MEA arose through duplication of an ancestral E(Z) homologue within the Brassicaceae family, indicating that MEA function came about relatively recently in angiosperm evolutionary history (Fig. 1) (Spillane et al., 2007).

SWN, the third E(Z)-like protein in Arabidopsis, also appears to participate in trimethylation of H3K27 at loci important for flower development, including *AGAMOUS* and *SHOOTMERISTEMLESS* (*STM*)(Katz et al., 2004; Schubert et al., 2006). MEA, CLF and SWN are probably responsible for the regulation of many genes and it was shown that more than 4000 genes carry H3K27 trimethylation marks (Makarevich et al., 2006; Zhang et al., 2007a). The specific role of each of them might potentially be identified through genome-wide comparisons of histone modifications in wild-type plants and *mea*, *clf* and *swn* mutants, and it should be considered that CLF and SWN regulate genes involved in many different processes of the plant life cycle.

B. CLASS II HKMT ENZYMES

Class II HKMTs are essentially implicated in the methylation of H3K36 (Fig. 3), a chromatin modification that is enriched within the region of actively transcribed genes (Lee and Shilatifard, 2007). Except for SDG4, class II HKMTs have a SET domain preceded by an AWS (Associated with SET) motif (Ng et al., 2007). The function of the AWS motif is unknown, but it is also found in mammalian class II HKMTs.

Functional insights have been obtained for two class II HKMTs: SDG4 and SDG8. Mutation of SDG8 affects FLC expression and induces an early flowering phenotype (Zhao et al., 2005). SDG8 is implicated in H3K36 di- and trimethylation, but *sdg8* mutations do not affect H3K4, H3K9 or H3K27 methylation at the *FLC* locus (Zhao et al., 2005). Thus, changes in H3K36 methylation are sufficient to affect *FLC* expression. Several metabolic pathways are also perturbed in *sdg8* mutants (Cazzonelli et al., 2009; Dong et al., 2008; Xu et al., 2008; Zhao et al., 2005). *sdg8* mutants show altered expression of *SPS/BUS* (Supershoot/Bushy) and *UGT74E2* genes, both of which affect shoot branching, a key process for plant biomass and seed production (Dong et al., 2008). Expression of *CAROTENOID ISOMERASE*, a gene required for carotenoid synthesis, is also perturbed in

sdg8 mutants. Consequently, a lower accumulation of lutein is observed in *sdg8* mutants, a carotenoid implicated in photosynthesis and photoprotection (Cazzonelli et al., 2009).

SDG4 was recently shown to be involved in pollen and stamen development (Cartagena et al., 2008; Thorstensen et al., 2008). Deficiency of SDG4 leads to reduced expression of multiple genes, probably due to defects in H3K4 dimethylation and H3K36 trimethylation. The *sdg4* mutation also affects fertility (Cartagena et al., 2008).

C. CLASS III HKMT ENZYMES

Like other HKMTs, class III HKMTs have also been shown to be involved in flowering time regulation. Class III HKMTs consist of five Arabidopsis genes that encode homologues of Trithorax; they have therefore been named *A*rabidopsis *T*rithora*x*-like proteins 1-5 (ATX1-5) (Fig. 1) (Avramova, 2009). Class III proteins contain both SET and a post-SET domains, as well as PHD (plant homeodomain), PWWP (proline–tryptophane– tryptophane–proline), FYRN (F/Y-rich N-terminus) and FYRC (F/Y-rich C-terminus) domains (Fig. 2) (Alvarez-Venegas and Avramova, 2001). The PHD domain is thought to interact with trimethylated H3K4 (Peña et al., 2006). The PWWP domain is present in diverse proteins involved in chromatin function, including histone-modifying enzymes, DNA-modifying enzymes and transcription factors and have been found to interact with both histone and DNA (Laue et al., 2008;Qiu et al., 2002;Stec et al., 2000;Wang et al., 2009).

ATX1 and ATX2 form a protein sub-group: ATX1 mediates H3K4 trimethylation, whereas ATX2 mediates H3K4 dimethylation (Fig. 3) (Pien et al., 2008;Saleh et al., 2008). *atx1* mutants display an early flowering phenotype and altered leaf morphogenesis (Alvarez-Venegas et al., 2003;Saleh et al., 2008). Intriguingly, the double *atx1 atx2* mutant has an even more severe early flowering phenotype than *atx1*, suggesting that ATX1 and ATX2 activities overlap for proper expression of genes implicated in flowering time regulation (Pien et al., 2008;Saleh et al., 2008).

Despite the evidence for partial redundancy in controlling flowering time, ATX1 and ATX2 do not appear to regulate the same pool of genes (Saleh et al., 2008). Transcriptome analysis revealed that 7% of overall gene expression is affected in *atx1*. By contrast, only 0.7% of all genes display a different pattern of expression in *atx2* mutants compared with controls (Alvarez-Venegas et al., 2006).

To date, no functions have been reported for ATX3, ATX4 or ATX5. These proteins have very conserved amino acid sequences, suggesting that they may have redundant functions. Double and triple mutant combinations of these three genes might potentially reveal their functional significance in *A. thaliana*.

ATXR3 and ATXR7 also belong to the class III AtKMTs; however, no data are available on the putative role and/or activity of ATXR3. However, ATXR7 (also known as SDG25) is able to specifically methylate Histone 3 *in vitro* and loss of function of *SDG25* promotes flowering through reduction of *FLC* expression (Berr et al., 2009). Further analyses suggest that ATXR7 might be implicated in H3K36 dimethylation and its role might overlap with the class II HKMT, SDG26 (Berr et al., 2009).

D. CLASS IV HKMT ENZYMES

There are two class IV HKMTs in Arabidopsis, ATXR5 and ATXR6. Both proteins possess a PHD domain associated with their SET domain. Class IV proteins have an additional motif that allows them to interact with *p*roliferating *c*ell *n*uclear *a*ntigen (PCNA) (Raynaud et al., 2006). PCNA is a processivity factor for DNA polymerase delta during DNA replication, which suggests a role for class IV HKMTs in cell cycle regulation (Raynaud et al., 2006).

ATXR5 and ATXR6 were recently shown to carry out monomethylation of H3K27 (Fig. 3) (Jacob et al., 2009). ATXR5 and ATXR6 appear to act redundantly, because depletion of H3K27 monomethylation is only detectable in the *atxr5 atxr6* double mutant (Jacob et al., 2009). Genome-wide analyses have revealed the presence of H3K27 monomethylation in heterochromatic chromocentres, whereas H3K27 di- and trimethylation are mainly present in euchromatic regions (Jacob et al., 2009;Zhang et al., 2007a). This suggests that distinct H3K27 methylation states correlate with different chromatin states. Interestingly, derepression of repetitive elements occurs in *atxr5 atxr6* double mutants (Jacob et al., 2009) and is correlated with reduced H3K27 monomethylation but not reduced DNA methylation or H3K9 methylation, confirming a key role for H3K27 monomethylation in gene silencing and genome stability (Jacob et al., 2009;Mathieu et al., 2005).

IV. CLASS V HKMTS MARK INACTIVE CHROMATIN VIA HISTONE 3 LYSINE 9 METHYLATION

A. SUVH PROTEINS

1. Discovery of SUVH proteins—In 2002, two independent mutant screens revealed roles for SUVH4/KRYPTONITE in H3K9 methylation and gene silencing in Arabidopsis (Jackson et al., 2002; Malagnac et al., 2002). In these screens, *suvh4* mutations were identified by their effects on the expression of the *SUPERMAN* locus, thereby affecting the number of floral organs (Jackson et al., 2002), or by the derepression of silenced *PHOSPHOANTHRINILATE ISOMERASE* (*PAI*) genes (Bender, 2004b; Luff et al., 1999; Malagnac et al., 2002). Importantly, cytosine methylation patterns were also perturbed at the loci where H3K9 dimethylation was lost, revealing a link between histone methylation and DNA methylation (Jackson et al., 2002). In other studies, depletion of cytosine methylation in the methyltransferase mutant met1 correlates with altered H3K9 methylation (Tariq et al., 2003), further indicating a functional relationship between DNA methylation and H3K9 modification.

2. Characteristics of SUVH proteins—SUVH proteins have a SET domain, a pre-SET domain and a post-SET domain. An additional motif, named the *S*ET and *R*ING finger-*a*ssociated (SRA) domain, is also a characteristic of SUVH proteins (Fig. 2). There are 10 members of the SUVH protein family, which appears to be plant specific (Fig. 1).

The SRA domain serves as a methylcytosine-binding motif in both animals and plants (Citterio et al., 2004; Kraft et al., 2008; Unoki et al., 2004; Woo et al., 2007, 2008). In Arabidopsis, point mutations in the SRA domain of SUVH2 or SUVH4 leads to reduced H3K9 dimethylation, and deletion of the motif in SUHV4 or SUVH6 results in a failure of the protein to bind methylated DNA *in vitro* (Johnson et al., 2007). Interestingly, different SRA domains preferentially bind methylated cytosines, in particular, DNA sequence contexts. *In vitro*, the SUVH2 SRA domain has highest affinity for symmetric, CG methylation, whereas the SUVH9 SRA domain has highest affinity for asymmetric, CHH methylation (Johnson et al., 2008). HKMT binding to DNA sequences displaying different cytosine methylation contexts would provide an elegant mechanism for transducing epigenetic information encoded by DNA methylation patterns into altered histone methylation states.

3. Activity of SUVH proteins—The H3K9 methyltransferase activity of SUVH4 was first inferred from molecular genetic studies, and then confirmed by mass spectrometric analysis of *in vitro* methylated histones (Jackson et al., 2002, 2004; Johnson et al., 2004; Malagnac et al., 2002). *In vitro* activities for SUVH1, SUVH5 and SUVH6 indicate that these proteins are H3K9 methyltransferases, which raises the possibility that all SUVH

proteins methylate H3K9 (Fig. 3) (Ebbs and Bender, 2006; Ebbs et al., 2005; Naumann et al., 2005).

SUVH2 and SUVH9 arose via a recent duplication in the Arabidopsis genome (Blanc et al., 2000, 2003). Initial evidence suggested that SUVH2 possesses H4K20 and H3K9 methyltransferase activity (Naumann et al., 2005). However, a more recent study found that *suvh2* and *suvh9* mutants, and *suvh2 suvh9* double mutants do not display detectably altered histone methylation patterns (Johnson et al., 2008). Moreover, unlike SUVH4, SUVH5 or SUVH6, no HKMT activity was detected for SUVH2 or SUVH9 *in vitro* (Johnson et al., 2008). Furthermore, the methyl group donor AdoMet does not bind to recombinant SUVH2 or SUVH9, whereas AdoMet binding to recombinant SUVH4, SUVH5 and SUVH6 is observed (Johnson et al., 2008). One possibility is that the biological functions of SUVH2 and SUVH9 depend primarily on SRA domain interactions with methylated DNA, rather than on putative HKMT activity.

4. Functions of SUVH proteins—SUVH4 is involved mainly in maintenance of CHG methylation controlled by the cytosine methyltransferase CMT3 (chromomethylase 3), such that a loss of DNA methylation is observed in *suvh4* mutants (Jackson et al., 2002; Malagnac et al., 2002). Jackson et al. (2002) showed that CMT3 does not interact with H3K9 dimethylation directly, and suggested that LHP1 was necessary to link SUVH4 and CMT3 activities (Jackson et al., 2002). However, subsequent work cast doubt on this hypothesis (Malagnac et al., 2002). It is now clear that *SUVH4* is responsible for the majority of H3K9 dimethylation in heterochromatin (Jackson et al., 2004; Jasencakova et al., 2003; Johnson et al., 2002). Mutations in SUVH4 do not lead to a significant reactivation of repetitive elements, as is observed for cytosine methyltransferase mutants. Several HKMT proteins may act redundantly to silence these loci. Indeed, SUVH5 and SUVH6 have been shown to work together with SUVH4 to silence inverted repeats (Ebbs and Bender, 2006; Ebbs et al., 2005). Moreover, the triple mutant *suvh2 suvh4 suvh9* shows altered expression of an F-box gene *SUPPRESSOR* of *DRM1 DMR2 CMT3* (*SDC*), which induces a curly leaf phenotype, whereas no changes are observed in single mutants (Johnson et al., 2008). Over-expressing *SUVH2* induces a general increase in heterochromatization mediated by an increase in repressive histone marks, including H3K9 dimethylation, H3K27 di- and trimethylation and H4K20 dimethylation (Naumann et al., 2005). This chromatin re-organization results in development changes, such as delayed leaf senescence (Ay et al., 2008).

The function of SUVH proteins in mediating H3K9 methylation is linked to cytosine methylation. A close correlation between these two heterochromatic marks is observed genome-wide (Bernatavichute et al., 2008; Gendrel et al., 2002). Furthermore, similar molecular defects are observed in mutants deficient in DNA methyltranferases or histone methyltransferases (Cao et al., 2003; Chan et al., 2006; Ebbs and Bender, 2006; Ebbs et al., 2005; Jackson et al., 2002; Johnson et al., 2007, 2008; Malagnac et al., 2002). For example, the triple mutant *drm1 drm2 cmt3* shows depletion of DNA methylation at CHG and CHH sites, as well as some reduction in CG methylation. A decrease in H3K9 dimethylation is also observed in *drm1 drm2 cmt3*, similar to the effect of *suvh4* mutations (Johnson et al., 2007). Similarly, *suhv4 suvh2 suvh9* and *drm1 drm2 cmt3* triple mutants display similar derepression of the SDC locus that correlates with a loss of DNA methylation and H3K9 methylation (Johnson et al., 2008).

Collectively, the available data reveal an important role for SUVH proteins in regulating the activity of loci present both in euchromatic and heterochromatic regions of the Arabidopsis genome. A recent study also analysed telomere length in the *suvh4* mutant. Compared to

wild-type plants, *suvh4* plants have shorter telomeres, which suggest that heterochromatin maintenance affects telomere stability (Grafi et al., 2007).

B. SUVR PROTEINS

1. Characteristics of SUVRs—Few studies have investigated the properties and functions of SUVR (SU (VAR)3–9 related) proteins. Like SUVH proteins, SUVR proteins have a SET domain which is associated with a pre-SET domain and a post-SET domain (Baumbusch et al., 2001; Thorstensen et al., 2006). However, in contrast to SUVH proteins, SUVRs lack an SRA domain. A novel N-terminal plant-specific domain, named WIYLD based on conserved residues, has been identified in SUVR1, SUVR2 and SUVR4 proteins (Fig. 2) (Thorstensen et al., 2006). The WIYLD domain is a conserved region (residues 21–77 in SUVR4) that possesses structural similarity to the C-terminal domain of RuvA, a DNA-binding protein implicated in homologous recombination in bacteria (Rice et al., 1997; Thorstensen et al., 2006). Five genes encoding SUVR proteins (SUVR1 to SUVR5) are present in the *A. thaliana* genome (Fig. 2). SUVR4 possesses H3K9 mono- and dimethylation activities, which suggests that this class of HKMTs is responsible for repressive chromatin marks (Fig. 3) (Thorstensen et al., 2006).

2. Functions of SUVR proteins—The functions of SUVR proteins remain unclear. However, SUVR1, SUVR2 and SUVR4 proteins are most similar to HKMTs in humans that are implicated in heterochromatin formation. A recent report found that Arabidopsis SUVR5 (also known as AtCZS) interacts with the remodelling factor AtSWP1 and that both SUVR5 and AtSWP1 are required to down-regulate *FLC* expression. This repression correlates with H3K9 dimethylation and H3K27 dimethylation at the *FLC* promoter (Krichevsky et al., 2007). As yet, no functions have been described for the other four SUVR proteins.

SUVR proteins all display at least partial nucleolar localization, which has not been observed for other classes of HKMTs (Thorstensen et al., 2006). The nucleolus is best known as the site of ribosome biogenesis, but it is now clear that myriad aspects of RNA metabolism occur in the nucleolus (Boisvert et al., 2007), including processing of siRNAs involved in RNA-directed DNA methylation (RdDM) (Li et al., 2006; Pontes et al., 2006). This nucleolar processing centre generates heterochromatic, 24-nt small RNAs that target both RdDM and H3K9 methylation to specific genomic regions. The presence in the nucleolus of SUVR proteins that catalyse H3K9 methylation could reflect a role in rRNA gene modification or a potential link to the heterochromatic siRNA production machinery.

V. CONCLUSIONS AND PERSPECTIVES

The analysis of Arabidopsis HKMTs remains challenging because of the large number of genes in this family. Of the 31 predicted histone HKMTs, half have assigned functions or lysine specificities (Fig. 3). Because different HKMTs can help activate or silence gene expression, they can have antagonistic roles in modulating gene activity. For instance, CLF is implicated in H3K27 methylation, a repressive chromatin mark, whereas ATX1 methylates H3K4, a mark of active chromatin. CLF and ATX1 can both modify the same chromatin region, with opposing effects on gene activity. Single *clf* and *atx1* mutants show abnormal leaf development, which is rescued in the double mutant *clf atx1*, indicating that this antagonism is biologically significant (Saleh et al., 2007).

Much effort is now focused on discovering specific functions for HKMTs. There are already several examples of HKMTs that seem to act preferentially at a specific tissue and time in development. An example is MEA, which is only expressed in the endosperm and the embryo (Grossniklaus et al., 1998; Kinoshita et al., 1999). Creating double and triple mutants for members of each Arabidopsis HKMT sub-group could reveal functions, and

functional redundancies, among these proteins. However, this is a time-consuming approach that requires loss-of-function mutations for all *HKMT* genes, which are not yet available for all of the genes. Artificial microRNAs that target one or more genes simultaneously might be a useful strategy for analysing the functions of potentially redundant HKMTs (Schwab et al., 2006). An important consideration for the interpretation of *in vivo* data concerning HKMT functions is that the enzymes may modify non-histone targets that are responsible for the observed phenotypes. For example, the plant protein Rubisco is targeted by a methyltransferase that possesses a SET domain (Trievel et al., 2002; Ying et al., 1999). Moreover, non-histone targets of HKMTs have been described in mammals, including transcription factors (Chuikov et al., 2004; Kouskouti et al., 2004).

Genome-wide DNA methylation and histone modification data have revealed heterogeneity in H3K9 dimethylation distribution across the genome. H3K9 dimethylation tends to occupy larger regions in pericentromeric regions than it does in the chromosome arms, suggesting that distinct H3K9 methyltransferases, possibly the SUVR and SUVH proteins, could regulate the level of histone methylation in these different regions (Bernatavichute et al., 2008). Moreover, a genome-wide analysis of H3K27 trimethylation patterns also revealed that perhaps 4400 *A. thaliana* genes are impacted by this specific type of histone modification (Zhang et al., 2007b). This observation is consistent with the hypothesis that H3K27 methylation is a major silencing mechanism in plants. It is known that H3K27 and H3K9 methylation function independently as repressive chromatin marks (Mathieu et al., 2005). Recent data obtained for class IV HKMTs ATXR5 and ATXR6 confirm this hypothesis (Jacob et al., 2009).

H3K9 methylation and DNA methylation are both critical for epigenetic regulation of gene expression in plants, but the mechanisms linking these interdependent processes remain to be fully determined. However, the SRA domain of SUVH proteins helps explain how H3K9 methyltransferase activity can be recruited to regions characterized by methylated DNA, as does the presence of a chromodomain in the DNA methyltransferase (cf. SUVHs proteins). CMT3 can potentially explain the recruitment of DNA methyltransferase activity to nucleosomal regions enriched for histones methylated on H3K9 or H3K27.

Intriguingly, neither SUVH2 nor SUVH9 displays HKMT activity but both can interact with methylated DNA. Alignment of SUVH proteins reveals amino acids substitutions in the SUVH2 and SUVH9 SET domains that might explain their lack of activity. However, they retain conserved pre-SET, SET and post-SET domains. It would be interesting to test whether their SET domains can interact with methylated H3K9. If this were the case, it might be possible that SUVH2 and SUVH9 recognize methylated DNA, and, at the same time, protect methylated H3K9 from histone lysine demethylase activities.

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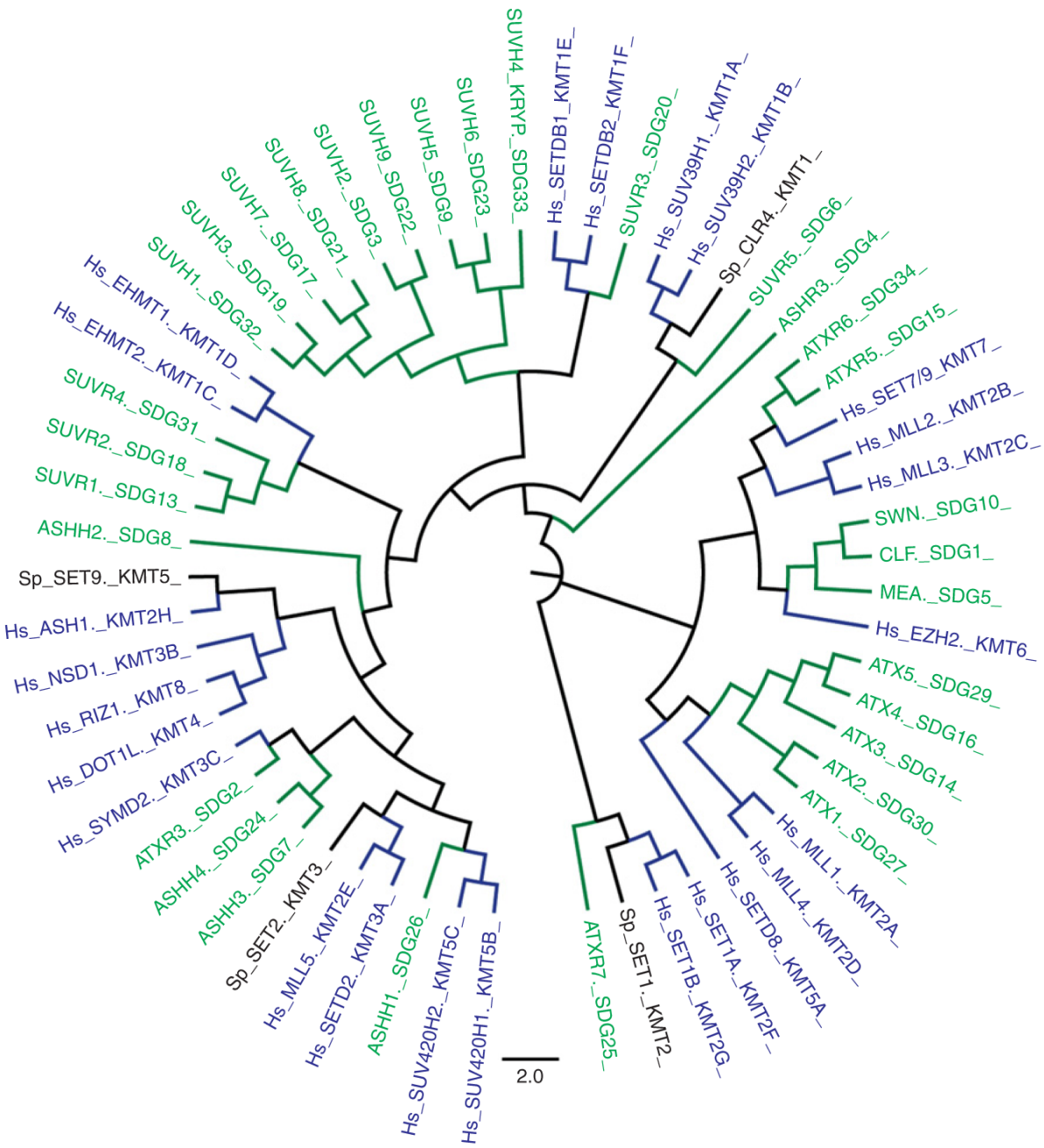

Fig. 1.

HKMTs organization and evolution. Relationships among HKMT Arabidopsis HKMT (grey), Human (Hs) HKMT and fission yeast (Sp) HKMT (dark) protein sequences determined by clustalW multiple alignment, followed by Neighbour Joining and Boostrap analysis.

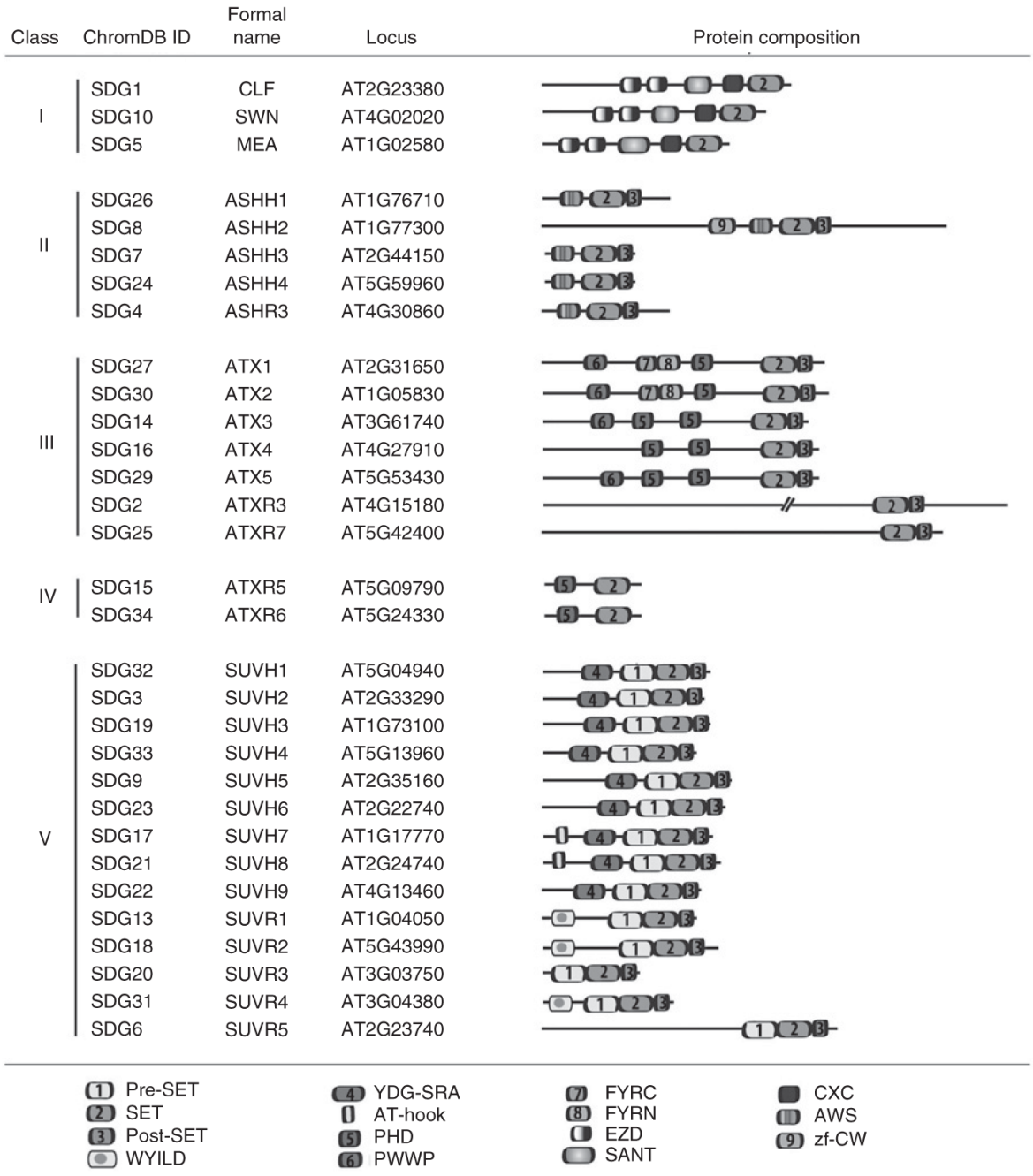

Fig. 2.

Domain architecture of histone HKMTs in *A. thaliana*. Abbreviations: EZD, E(Z) domain; SANT, SWI3, ADA2, N-CoR and TFIIIB" DNA-binding domain; CXC, cysteine-rich region; PHD, plant homeodomain; zf-CW, a zinc finger with conserved Cys and Trp residues; PWWP, domain named after a conserved Pro–Trp–Trp–Pro motif; FYRN, F/Y-rich N-terminus; FYRC, F/Y-rich C-terminus.

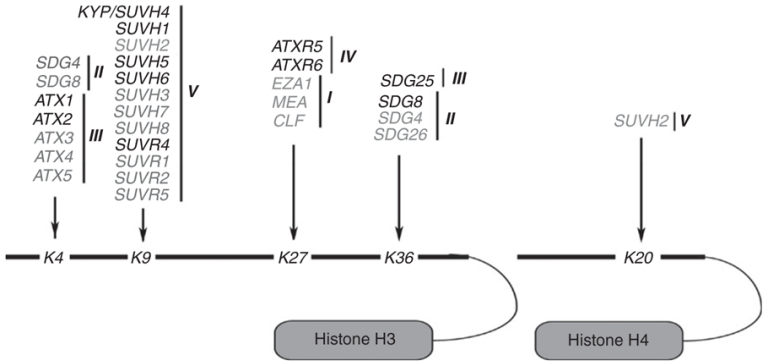

Fig. 3.

Diagram representing the H3 and H4 Lysines targeted by Arabidopsis HKMT proteins. The HKMTs whose activity has been biochemically demonstrated are highlighted using in black, whereas the HKMTs whose specificities have not been confirmed are shown in gray.