Structural and Functional Coordination of DNA and Histone Methylation

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SUMMARY

One of the most fundamental questions in the control of gene expression in mammals is how epigenetic methylation patterns of DNA and histones are established, erased, and recognized. This central process in controlling gene expression includes coordinated covalent modifications of DNA and its associated histones. This article focuses on structural aspects of enzymatic activities of histone (arginine and lysine) methylation and demethylation and functional links between the methylation status of the DNA and histones. An interconnected network of methyltransferases, demethylases, and accessory proteins is responsible for changing or maintaining the modification status of specific regions of chromatin.

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Outline

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OVERVIEW

All cells face the problem of controlling the amounts and timing of expression of their various genes. In some cases, this control involves relatively long-term and heritable modifications to the chromatin, albeit nonpermanent. Such modifications that do not change the DNA sequence are referred to as "epigenetic." The resulting epigenetic effects maintain the various patterns of gene expression in different cell types. Epigenetic modifications include DNA methylation and histone posttranslational modifications (PTMs).

Nucleosomes consist of ~146 bp of DNA wrapped approximately 1.8 times around a histone octamer and are evolutionarily conserved across all eukaryotes. The combinatorial pattern of DNA and histone modifications constitutes an epigenetic code that shapes transcriptional patterns and genomic stability. The coding modification is "written" by sequence- and site-specific enzymes, and "interpreted" or "read" by effector (or reader) molecules that mediate the assembly of higher-order chromatin structures, involving remodeling complexes, histone variants, and noncoding RNAs (see articles by Becker and Workman 2013; Allis et al. 2014; Henikoff and Smith 2014). Representative enzymes responsible for histone methylation (SET domain proteins, Dot1, and protein arginine methyltransferases), histone lysine demethylation (LSD1 and Jumonji proteins), and DNA methylation (Dnmt1 and Dnmt3) are discussed in this article. DNA methylation is an epigenetic modification that has long been known to repress transcription. Histone methylation, depending on the histone and residue of modification, contributes to either active or repressive chromatin configurations. The functional implications of the structural determination of enzymes in linking histone modifications to that of DNA in mammalian cells are also discussed. This article is complemented by subsequent articles discussing the structure and function of proteins that read DNA and histone methylation (Patel 2014), and previous articles discussing histone acetylation writers (HATs), histone deacetylation (HDAC) "eraser" enzymes, and readers of these epigenetic marks (Marmorstein and Zhou 2014; Seto and Yoshida 2014).

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1 HISTONE METHYLATION AND DEMETHYLATION

1.1 Histone Lysine (K) Methyltransferases (HKMTs)

All known HKMTs (referred to in some of the literature as PKMTs or KMTs, as many of the substrates are nonhistone proteins) contain an evolutionarily conserved SET domain comprised of 130 amino acids (reviewed in Cheng et al. 2005), except Dot1 (see Sec. 1.2). The SET domain was first identified as a shared sequence motif in three *Drosophila* proteins: suppressor of variegation (Su(var)3-9), enhancer of zeste (E(z)), and homeobox gene regulator trithorax (Trx; Jenuwein et al. 1998). Mammalian homologs of the *Drosophila* Su(var)3-9 protein, SUV39H1 in humans, and Suv39h1 in mouse were the first characterized HKMTs involved in H3K9 methylation (Fig. 1A) (Rea et al. 2000). Since then, more than 50 SET domain-containing proteins have been identified in humans, with a proven or predicted enzymatic role in performing lysine methylation on histone tails (reviewed in Volkel and Angrand 2007).

The majority of the SET-containing HKMTs contain at least one additional protein module in their protein sequence (e.g., a chromodomain in SUV39H1; Fig. 1A). SET-containing HKMTs are grouped into six different subfamilies based on sequence homology within and around the catalytic SET domain, homology with other protein modules, and their structures. The six subfamilies include SET1, SET2, SUV39, EZH, SMYD, and PRDM (Volkel and Angrand 2007). A number of SET-containing HKMTs, however, do not fall into the above six subfamilies because of lacking sequences or conservation flanking their SET domains. Examples of such proteins include Set8 (also known as PR-Set7; Couture et al. 2005; Xiao et al. 2005), which monomethylates H4K20 (H4K20me1), and SUV4-20H1 and SUV4-20H2, which di- and trimethylate H4K20 (H4K20me2 and me3). Set7/9 can monomethylate H3K4 (H3K4me1; Xiao et al. 2003) and many other nonhistone substrates, whereas SetD6 monomethylates only the nonhistone substrate, RelA, a subunit of NF-κB (Levy et al. 2011).

Structures of many SET domains from different subfamilies have been solved in various combinations, including bound to peptide substrates and methyl donor (*S*-adenosyl-L-methionine, also known as AdoMet or SAM) or reaction product (*S*-adenosyl-L-homocysteine, also known as AdoHcy or SAH). The SET domain adopts a unique structure formed by a series of β-strands folded into three sheets surrounding a knot-like structure (Fig. 1B). The knot-like structure (or pseudoknot) is formed by the carboxyl-terminal segment of the SET domain, which passes through a loop formed by the preceding stretch of sequences. Formation of this pseudoknot structure brings the two conserved SET domain sequence motifs, III and IV (Fig. 1C), in close proximity to the AdoMet-binding region and peptide-binding channel (Fig. 1D).

Available crystal structures of the SUV39 HKMT subfamily, which methylates H3K9 (DIM-5, Clr4, GLP/EHMT1, G9a/EHMT2, and SUV39H2), show the presence of two closely packed cysteine-rich modules in both the pre-SET and post-SET (before and after the SET) domains (Fig. 1E). These two modules are important for maintaining structural stability (pre-SET) and forming part of the active site lysine channel (post-SET) (Zhang et al. 2002; Zhang et al. 2003). The pre-SET module contains nine conserved cysteines, which coordinate three Zn^{2+} atoms in a triangular geometry. The post-SET module contains three conserved cysteines, which along with a cysteine from the conserved motif III sequence, (R/H)F(I/V)NHxCxPN, tetrahedrally coordinate the fourth Zn^{2+} atom near the active site. Binding of the fourth Zn^{2+} at the active site is essential for the activity of the SUV39 subfamily (Zhang et al. 2003).

1.2 Dot1p: Non-SET Domain HKMT

Histone H3 Lys-79 (H3K79) is methylated by Dot1p (reviewed in Frederiks et al. 2011), a protein originally identified as a disruptor of telomeric silencing in *Saccharomyces cerevisiae* (Singer et al. 1998). Methylation of H3K79 in *S. cerevisiae* is important for the proper localization of the silent information regulator complex and DNA damage signaling (see Grunstein and Gasser 2013).

Dot1p is a Class-I methyltransferase, as suggested by the presence of the AdoMet-binding sequence motifs (Dlakic 2001; Schubert et al. 2003), similar to those found in protein arginine methyltransferases and DNA methyltransferases (see Secs. 1.3 and 2). Class-I methyltransferases such as Dot1p are distinct from most other HKMTs because they do not contain the SET domain. Thus, they have an entirely different structural scaffolding and unrelated local active-site spatial arrangement that catalyzes AdoMet-dependent methyl transfer to a protein lysine side chain.

Yeast Dot1p contains a core region (indicated in Fig. 2A) conserved between Dot1p homologs in human, *Caenorhabditis elegans*, *Drosophila*, and the mosquito, *Anopheles gambiae*. The length of these Dot1 proteins varies from 582 amino acids in yeast to 2237 amino acids in *Drosophila*. The conserved Dot1p core is located at the carboxyl terminus in yeast, but is at the amino terminus in human, *C. elegans*, *Drosophila*, and *Anopheles gambiae* Dot1p homologs. The Dot1p conserved core contains an amino-terminal helical domain and a seven-stranded catalytic domain that harbors the binding site for the methyl donor and an active-site pocket sided with conserved hydrophobic residues (Fig. 2B).

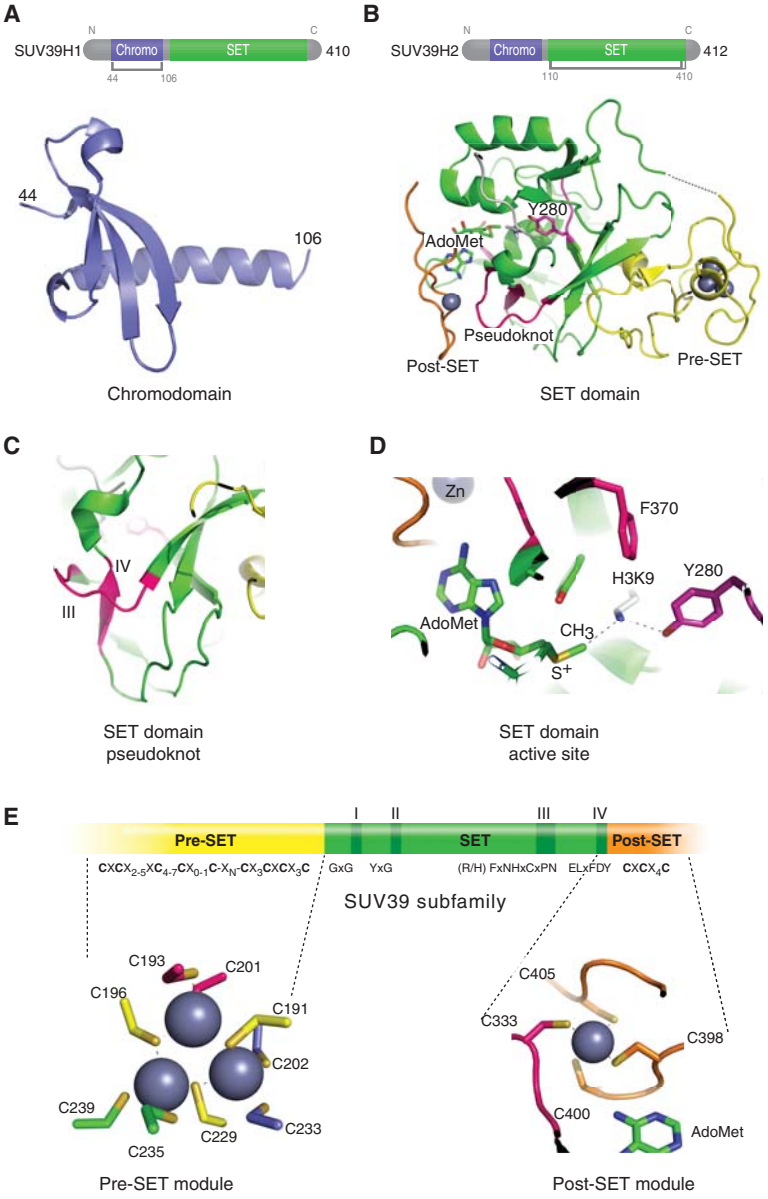

Figure 1. Structural features of SUV39H1 and SUV39H2. (*A*) Ribbon diagram of amino-terminal chromodomain of SUV39H1 (PDB 3MTS). (*B*) Carboxyl-terminal SET domain structure of SUV39H2 (PDB 2R3A). (*C*) Formation of the pseudoknot by motifs III and IV. (*D*) Formation of the active site showing the methyl donor (*S*-adenosyl-L-methionine [AdoMet]), target H3K9 lysine, catalytic Y280 residue, and F370 Phe/Tyr Switch (Collins et al. 2005). AdoMet-dependent methyltransferases (including HKMTs) share a reaction mechanism in which the nucleophile acceptor (NH_2) attacks the electrophilic carbon of AdoMet in an S_N2 displacement reaction. (*E*) SUV39H1 and H2 have a pre-SET segment containing nine invariant cysteines, the SET region containing four signature motifs, and the post-SET region containing three invariant cysteines. An enlargement of the pre-SET Zn_3Cys_9 triangular zinc cluster structure is illustrated on the *bottom left* and the post-SET zinc center on the *bottom right* panel.

Dot1p has several unique biochemical properties. Yeast Dot1p and its human homolog Dot1L methylate only nucleosomal substrates, but not free histone H3 protein (reviewed in Frederiks et al. 2011). A stretch of positively charged residues (i.e., Lys-rich) at the carboxyl-terminal end of the human Dot1L core or the amino-terminal end of the yeast Dot1p core were critical for nucleosome binding (Fig. 2C) and therefore for enzymatic activity (Min et al. 2003; Sawada et al. 2004; Oh et al. 2010). Given in *S. cerevisiae*, H3K79 methylation requires ubiquitination of H2B K123 in vivo (Briggs et al. 2002), and both histone residues are located on the same nucleosome disk surface

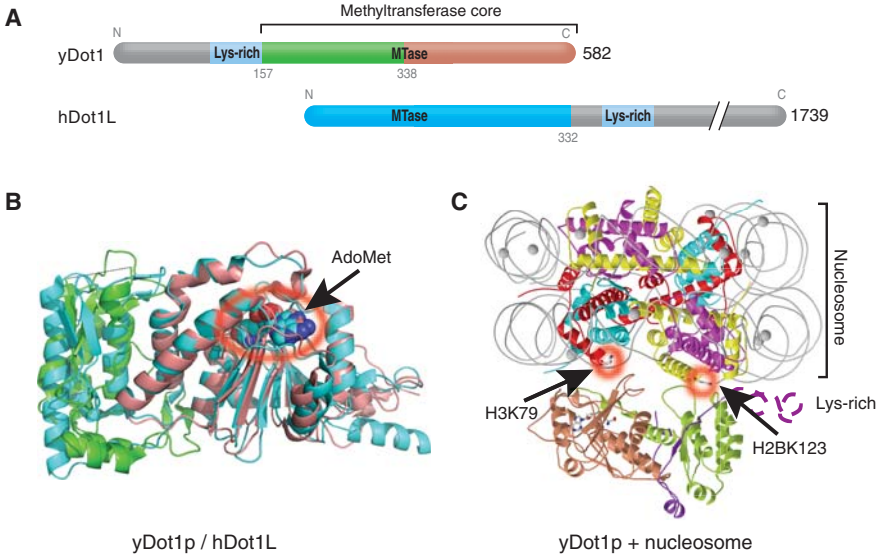

Figure 2. Dot1p family (non-SET HKMTs). (*A*) Schematic representation of Dot1 homologs from yeast and human, indicating the conserved methyltransferase core regions. (*B*) Superimposition of the conserved core regions of yeast Dot1p (residues 176-567; PDB 1U2Z), colored in green and brown, and human Dot1L (residues 5-332; PDB 1NW3), colored in cyan. The amino-terminal helical domains are shown on the *left* side of the panel in green for yDot1p or cyan for hDot1L. The carboxyl-terminal catalytic domain is shown on the *right* side of the panel with the bound methyl donor, AdoMet, as spheres (circled in red with an arrow). (*C*) A model of yDot1p docked with a nucleosome, adapted from Sawada et al. (2004). The structure of the nucleosome core particle is shown as ribbons (red, H3; green, H4; magenta, H2A; yellow, H2B; gray lines, DNA). The model was put together by aligning the target H3K79, located on the nucleosome disk surface, with the active site pocket of Dot1p.

~30 Å apart, Dot1p may interact specifically with nucleosomes containing ubiquitinated H2B (Fig. 2C) (Oh et al. 2010). Such an interaction could be significant in vivo because Dot1p could be recruited to specific high-order chromatin in which ubiquitinated histone H2B might serve as a spacer between adjacent nucleosome disk surfaces (Sun and Allis 2002), allowing Dot1p access to its target H3K79 lysine (see also Fig. 10 in Allis et al. 2014).

Mistargeted hDOT1L function has been implicated in the leukemogenesis of mixed lineage leukemia with the MLL-AF10 fusion (Okada et al. 2005). It does this by interacting with the AF10 protein and up-regulating genes such as Hox9a. Recently, the in vivo inhibition of hDot1L led to the increased survival of mice that had a xenograft model of mixed lineage leukemia (Daigle et al. 2011). This is, notably, the first example of selective inhibition of an HKMT that has efficacy in a cancer model.

1.3 Protein Arginine Methylation

Protein arginine methylation is a common posttranslational modification in eukaryotes. There are two major types of protein arginine (R) methyltransferases (PRMTs) that transfer the methyl group from AdoMet to the guanidino group of arginines in protein substrates (Lee et al. 1977), called type I and type II PRMTs (Fig. 3A). Both catalyze the formation of monomethylarginine (Rme1) as an intermediate, but type I PRMTs also form asymmetric dimethylarginine (Rme2a), whereas type II PRMTs form symmetric dimethylarginine (Rme2s). Among the nine canonical members of PRMT family (Herrmann et al. 2009; Table 1), only PRMT5 (also known as JBP1 for Jak-binding protein 1; Branscombe et al. 2001), and possibly PRMT7 and PRMT9 are type II PRMTs (Lee et al. 2005; Cook et al. 2006); they symmetrically dimethylate-specific arginines not only on histones, but also other proteins such as myelin basic protein (Kim et al. 1997), spliceosomal Sm proteins (Friesen et al. 2001), and Piwi proteins (Vagin et al. 2009). Highly relevant to the focus of this article, PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, the de novo DNA methyltransferase, coupling histone arginine, and DNA methylation in gene silencing (Zhao et al. 2009).

The PRMT proteins vary in length from 353 amino acids for PRMT1 to 637 amino acids for PRMT5/JBP1, but they all contain a conserved core region of approximately 310 amino acids (Fig. 3B). The sequences beyond the conserved PRMT core region are all amino-terminal additions; however, PRMT4 also has a carboxyl-terminal addition. The size of the amino-terminal additions varies

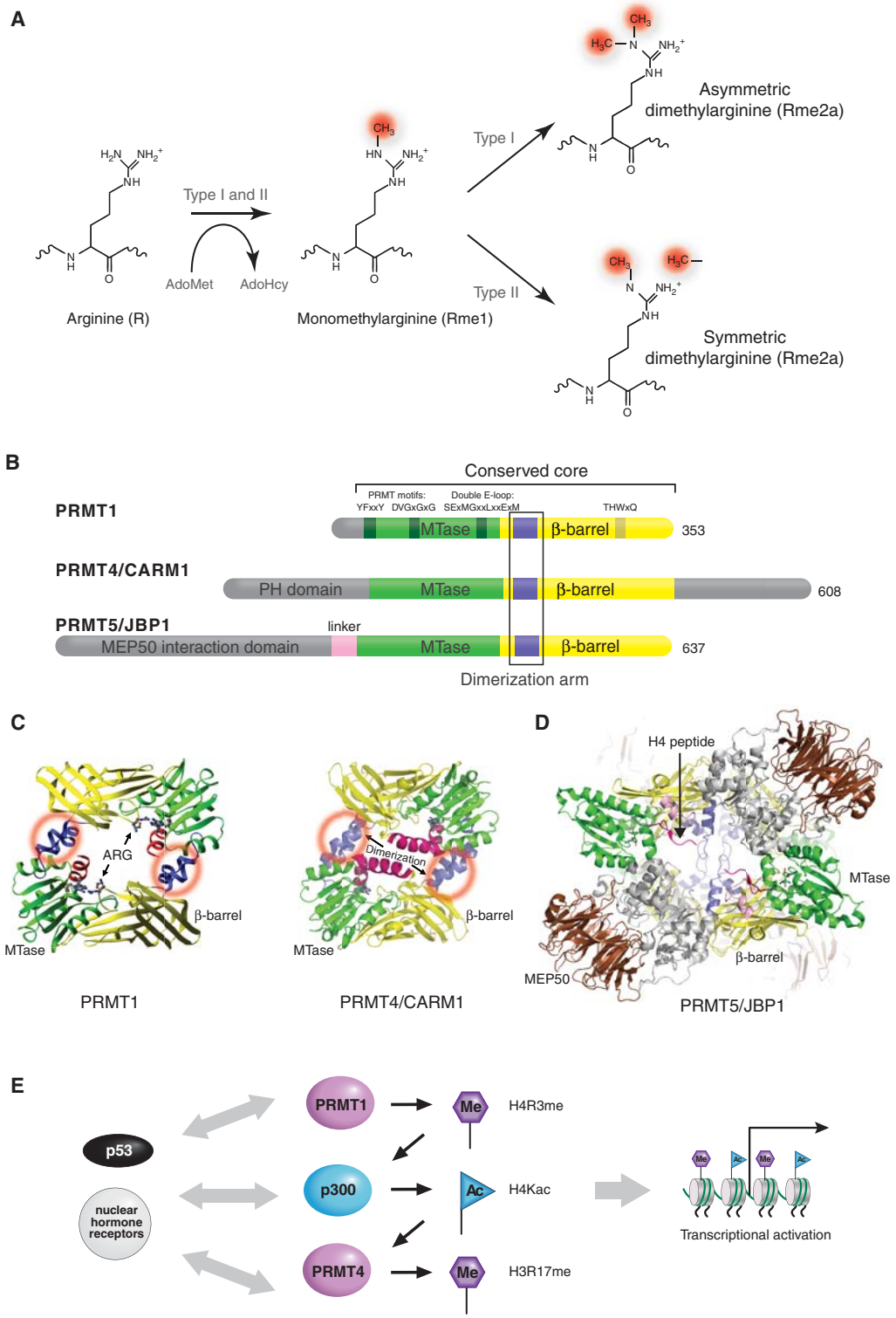

Figure 3. PRMT family. (*A*) Reactions catalyzed by the two major types of protein arginine methylation. (*B*) Representatives of PRMT members (type I: PRMT1 and PRMT4; type II: PRMT5). The conserved methyltransferase (MTase) domain is in green and the unique β-barrel domain in yellow. (*C*) Dimeric structures of PRMT1 (PDB 1OR8) and PRMT4 (PDB 3B3F). Dimerization arms are indicated by red circles. (*D*) Type II PRMT5-MEP50 tetramer complex (DPB 4GQB), formed by the stacking of two dimers. The second dimer is faded in the background. MEP50 (colored in brown) interacts with the amino-terminal domain (gray) of PRMT5. Bound H4 peptide is colored in red. (*E*) An example of three coactivators acting synergistically for p53-mediated transcription.

Table 1. Members of human PRMT family

Enzyme	Type	Activity	Chromosome	EST	Coding exon	Genomic size (kb)	Protein accession number	Protein size (residues)
PRMT1	I	+++	19q13	+++	9–10	10	CAA71764	361
PRMT2	I	-	21q22	++	10	30	P55345	433
PRMT3	I	+	11p15	+	13	50	AAH64831	531
PRMT4(CARM1)	I	+	19p13	++	16	50	NP_954592	608
PRMT5(JBP1)	II	+	14q11	++	17	8.5	AAF04502	637
PRMT6	I	+	1p13	+/−	1	2.5	AAK85733	375
PRMT7	II?	+	16q22	++	17	41	Q9NVM4	692
PRMT8	I	?	12p13	+	9	52	AAF91390	334
PRMT9	II?	?	4q31	+	10	40	AAH64403	845

between ~30 amino acids in PRMT1 to ~300 amino acids in PRMT5. The variation in amino termini allows each PRMT to be subject to a different mode of regulation. An interesting feature of PRMT7 and PRMT9 is that they seem to have arisen from a gene duplication event and contain two conserved core regions, each with a putative AdoMet-binding motif (Miranda et al. 2004; Cook et al. 2006).

Several crystal structures of type I PRMTs are currently available (Cheng et al. 2005; Troffer-Charlier et al. 2007; Yue et al. 2007). These structures reflect a striking structural conservation of the PRMT catalytic core (Fig. 3B). The overall monomeric structure of the PRMT core can be divided into three parts: a methyltransferase domain, a β-barrel, and a dimerization arm. The methyltransferase domain has a consensus fold conserved in Class-I AdoMet-dependent methyltransferases (like that of Dot1p) that harbor an AdoMet-binding site (Schubert et al. 2003). The β-barrel domain is unique to the PRMT family (Zhang et al. 2000). Dimer formation is a conserved feature in the type I PRMT family, validated by crystal structure studies, an example of which is illustrated in Figure 3C (Cheng et al. 2005). Dimerization may be required to correctly engage the residues in the AdoMet-binding site, so that they bind AdoMet and/or generate dimethylated (Rme2) products processively, akin to the spread of H3K9 trimethylation by DIM-5 (Zhang et al. 2003). Indeed, phosphorylation of PRMT4 at a conserved serine residue in the dimer interface results in inefficient AdoMet binding, and reduced histone methylation activity (Higashimoto et al. 2007). Similarly, an allosteric inhibitor that binds in the dimer interface of PRMT3 results in reduced binding of AdoMet and methylation activity (Siarheyeva et al. 2012).

The type II enzyme, PRMT5, functions as part of various high molecular weight protein complexes that also contain the WD repeat protein MEP50 (methylosome protein 50) (Friesen et al. 2002). The structure of the human PRMT5-MEP50 complex revealed that PRMT5 was organized into a tetramer through the stacking of two primary dimers and the amino-terminal domain of PRMT5 interacting with the seven-bladed β-propeller MEP50 (Fig. 3D) (Antonysamy et al. 2012).

Two well-studied enzymes, PRMT1 and PRMT4, methylate histones H2B, H3, and H4 (reviewed in Bedford and Clarke 2009), in addition to many nonhistone substrates including the carboxyl-terminal domain of RNA polymerase II (Sims et al. 2011). Histone arginine methylation is a component of the "histone code" that directs a variety of processes involving chromatin. For example, methylation of H4R3 by PRMT1 facilitates H4 acetylation and enhances transcriptional activation by nuclear hormone receptors. It acts synergistically with PRMT4 because PRMT4 prefers acetylated histone tails to generate methylated H3R17 (Wang et al. 2001; Daujat et al. 2002). Similarly, the synergistic action in vitro of PRMT1, PRMT4, and p300 for p53-mediated transcription is the greatest when all three coactivators are present, whether added sequentially or at the same time (Fig. 3E) (An et al. 2004). Even preincubating a chromatin template with p53 and PRMT1 significantly stimulated the histone acetyltransferase activity of p300, as did preincubation of the template with p53 and p300 to stimulate H3 arginine methylation by PRMT4. Indeed, PRMT4 was initially discovered as a transcriptional coactivator-associated arginine (R) methyltransferase 1 (CARM1; Chen et al. 1999). PRMT4/CARM1 also acts synergistically with the p160 coactivator to stimulate gene activation by nuclear receptors (Chen et al. 1999; Lee et al. 2002). These results provide compelling evidence that histones are relevant targets for PRMT4/CARM1, PRMT1, and p300, and that the resulting histone modifications are directly important for transcription.

1.4 Lysine Demethylation by Oxidation: LSD1

To the surprise of the research community, protein lysine methylation was finally shown to be a reversible posttranslational modification in 2004 (described in Shi and Tsukada 2013). Before this, Bannister et al. had hypothesized that methyl groups from both lysine and arginine side chains

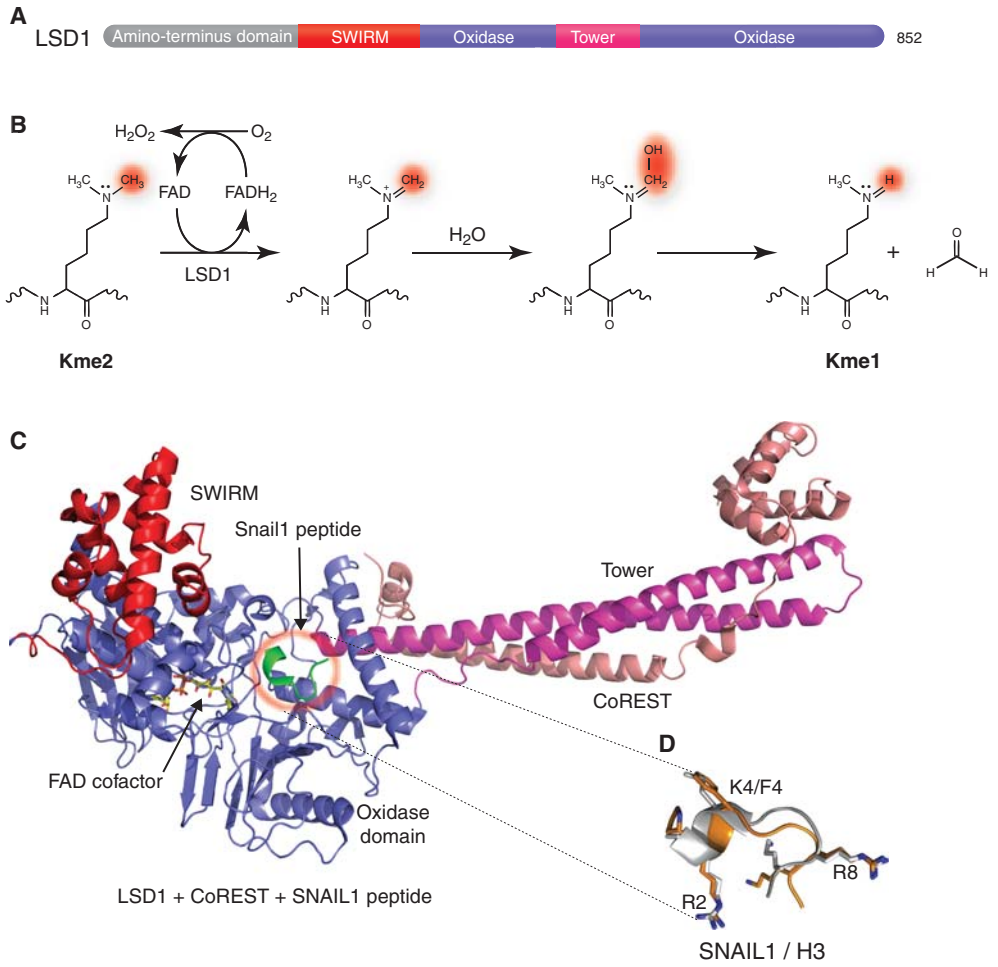

Figure 4. Histone demethylation by oxidation. (*A*) Schematic representation of human LSD1 domain organization: the amino-terminal putative nuclear localization signal, followed by a SWIRM (Swi3p, Rsc8p, and Moira) domain, and the catalytic oxidase domain. The oxidase domain contains an atypical insertion of the Tower domain not found in other oxidases. (*B*) Scheme of the demethylation reaction catalyzed by LSD1. (*C*) Crystal structure of LSD1-CoREST in complex with the SNAIL1 peptide (PDB 2Y48). LSD1 includes residues 171–836 in red, blue, and magenta. CoREST shows residues 308–440 in orange. The SNAIL1 peptide is in green, and the FAD cofactor is shown as a yellow ball-and-stick. (*D*) Superposition between SNAIL1 (orange) and histone H3 (gray) peptides. (Adapted from Baron et al. 2011).

could be oxidatively removed using a FAD (flavin adenine dinucleotide) cofactor as the electron acceptor (Bannister et al. 2002). Then came the discovery of the lysine-specific demethylase 1 (LSD1) protein (Fig. 4A) (Shi et al. 2004). LSD1 is a flavin-dependent amine oxidase that demethylates H3K4me2/me1 (Shi et al. 2004), H3K9me2/me1 in an androgen receptor-mediated pathway (Metzger et al. 2005), and even p53, a nonhistone protein (Huang et al. 2007). The closely related LSD2 demethylates H3K4me2/me1 (Karytinos et al. 2009). Both LSD1 and LSD2 demethylate methyl lysines by forming an imine intermediate that undergoes hydrolysis in aqueous buffer to complete the demethylation process (Fig. 4B). LSD1 and LSD2, however, cannot demethylate trimethylated lysines because of the mechanistic requirement for a protonated amine in this demethylation pathway.

Thus far, crystal structures of LSD1 in various configurations have been determined (Fig. 4C) (reviewed in Hou and Yu 2010). In one study, the first 16 residues of histone H3 was observed in a complex structure with LSD1-CoREST (Forneris et al. 2007), in perfect agreement with biochemical data that LSD1 is active on peptide substrates longer than 16 amino acids (Forneris et al. 2005). Interestingly, the amino-terminal extremity of the transcription factor SNAIL1 has sequence similarity with the amino-terminal tail of histone H3 (Lin et al. 2010), and binds to the LSD1 catalytic site in the same way as a histone H3 peptide substrate (Fig. 4D) (Baron et al. 2011). Specifically, the binding

positions of the amino-terminal Arg2, Phe4, and Arg7 residues of the SNAIL1 peptide correspond to the amino-terminal Arg2, Lys4, and Arg8 residues of histone H3. Thus, the Snail1-LSD1-CoREST complex effectively inhibits LSD1 enzymatic activity (Baron et al. 2011), and is found in certain cancer cells. The fact that LSD1 recognizes the first amino-terminal amino group (a conserved positive charge), customizing the orientation of the fourth amino acid side chain (i.e., H3K4me2/me1) to point toward and be in direct contact with the flavin ring of the cofactor, raises the question of how LSD1 demethylates methyllysines further away from the amino terminus (e.g., H3K9me2/me1), in an androgen receptor-dependent manner (Metzger et al. 2005).

1.5 Lysine Demethylation by Hydroxylation: Jumonji-Containing Demethylases

In search of enzymes capable of reversing methylated lysines, Trewick et al. hypothesized that Jumonji domain-containing Fe(II)- and α-ketoglutarate-dependent dioxygenases can reverse lysine methylation via a similar mechanism as the bacterial AlkB family of DNA repair enzymes (Fig. 5A) (Trewick et al. 2005). This hypothesis was quickly verified by the discovery of JHDM1 as the Jumonji domain-containing histone demethylase 1, using a biochemical assay based on the detection of formaldehyde, one of the predicted reaction products (Tsukada et al. 2006; described in Shi and Tsukada 2013). Jumonji-containing proteins are

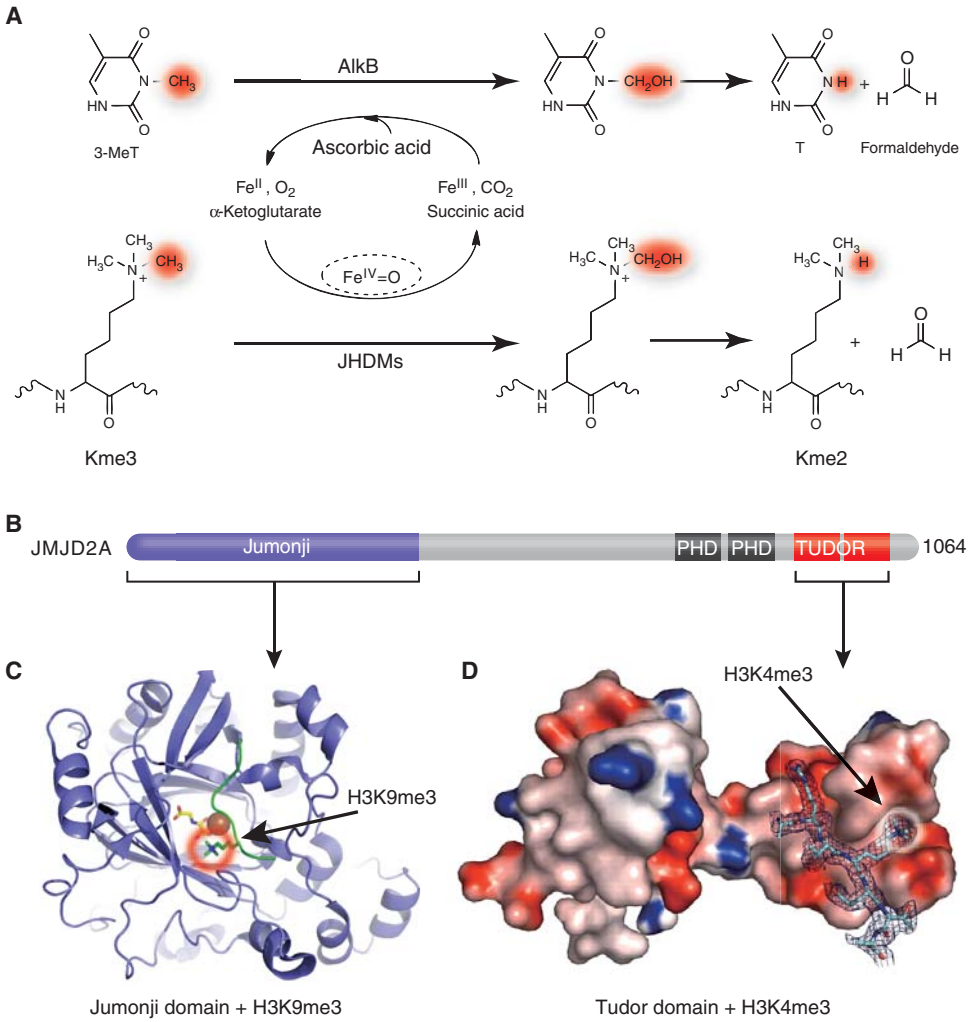

Figure 5. Demethylation by hydroxylation. (*A*) Mechanisms of demethylation of 3-methylthymine by AlkB (*top*) and of methyllysine by Jumonji-domain proteins (*bottom*). (*B*) Schematic representation of JMJD2A domain organization. (*C*) Structure of the amino-terminal Jumonji (ribbons) in complex with H3K9me3 (PDB 2OX0). (*D*) Structure of the carboxyl-terminal double Tudor domain (surface representation) in complex with H3K4me3 (PDB 2GFA). (Adapted, with permission, from Huang et al. 2006.)

members of the cupin super family (Clissold and Ponting 2001), including the Tet proteins involved in conversion of 5-methylcytosine to 5-hydroxymethylcytosine (Kriaucionis and Tahiliani 2014; Li and Zhang 2014). Demethylation reactions catalyzed by Jumonji enzymes follow a hydroxylation pathway involving a reactive Fe(IV) intermediate. As they do not require a lone pair of electrons on the target nitrogen atom, thereby they can demethylate mono-, di-, and trimethylated lysines (Fig. 5A) (Hoffart et al. 2006; Ozer and Bruick 2007).

JMJD2A contains carboxyl-terminal PHD and Tudor domains, which typically act as methyl-binding proteins, called readers, in addition to the amino-terminal Jumonji domain (Fig. 5B). The JMJD2A Jumonji domain alone is capable of demethylating H3K9me3/me2 and H3K36me3/me2. Structural studies revealed that the JMJD2A Jumonji domain predominantly recognizes the backbone of the histone peptides (unusual for a sequence-specific enzyme), which allows the enzyme to demethylate both H3K9 (Fig. 5C) and H3K36 (reviewed in Hou and Yu 2010; McDonough et al. 2010). The Tudor domain binds both H3K4me3 (Fig. 5D) and H4K20me3 (Huang et al. 2006; Lee et al. 2008). The functional connection between the methyl mark reader and eraser in JMJD2A, however, is currently not clear.

2 DNA METHYLATION

In mammals and other vertebrates, DNA methylation occurs at the C5 position of cytosine, generating 5-methylcytosine (5mC), mostly within CpG dinucleotides. This methylation, together with histone modifications, plays an important role in modulating chromatin structure, thus controlling gene expression and many other chromatin-dependent processes (Cheng and Blumenthal 2010; reviewed in Li and Zhang 2014). The resulting epigenetic effects maintain the various patterns of gene expression in different cell types (reviewed in De Carvalho et al. 2010). In mammals, DNA methyltransferases (Dnmts) include three proteins belonging to two families that are structurally and functionally distinct. Dnmt3a and Dnmt3b establish the initial CpG methylation pattern de novo, whereas Dnmt1 maintains this pattern during chromosome replication and repair (see Fig. 2 of Li and Zhang 2014).

2.1 Maintenance Methyltransferase Dnmt1

The so-called "maintenance" methyltransferase, Dnmt1, contains multiple functional domains (Fig. 6A) (Yoder et al. 1996). Structures are currently available for three amino-terminal deletions of mouse Dnmt1: an amino-terminal deletion of 350 residues (Δ350) in complex with AdoMet or its product AdoHcy (Takeshita et al. 2011), a larger deletion (Δ600) bound to DNA-containing an unmethylated CpG site (Song et al. 2011), and an even larger deletion (Δ730) bound to a hemimethylated CpG site (Song et al. 2012). When Dnmt1 is not bound to DNA, the amino-terminal replication focus targeting sequence (RFTS) domain of Δ350 is inserted into the DNA-binding surface cleft of the carboxyl-terminal MTase domain (Fig. 6B), indicating that this domain must be removed for methylation to occur. The RFTS domain is required for targeting Dnmt1 to replication foci, in which hemimethylated DNA is transiently generated. When the isolated RFTS domain is added in *trans* to an RFTS-lacking Dnmt1 protein, the RFTS domain acts as a DNA-competitive inhibitor of Dnmt1 (Syeda et al. 2011). In the structure of the Δ600 fragment, lacking the RFTS domain, the DNA bound CXXC domain (which specifically binds nonmethylated DNA) positions itself in the catalytic domain and prevents aberrant de novo methylation of CpG sequences (Fig. 6C). Only after physical removal of both the RFTS and CXXC domains can the carboxyl-terminal half of Dnmt1 (Δ730) bind to hemimethylated CpG DNA by flipping the target cytosine out of the double-stranded DNA helix into the active site (Fig. 6D). Thus, a multistep process, accompanied by structural changes, must occur during the targeting of full-length Dnmt1 to replication foci, which undergoes maintenance methylation of hemimethylated CpG DNA. This involves the removal of both RFTS and CXXC domains away from the catalytic center.

Dnmt1 alone, however, is insufficient for proper maintenance methylation. In vivo, maintenance methylation of hemimethylated CpG dinucleotides by Dnmt1 at DNA replication forks requires an accessory protein called UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1; Bostick et al. 2007; Sharif et al. 2007). UHRF1, a multidomain protein (Fig. 6E), binds both hemimethylated CpG site (Fig. 6F), the substrate of Dnmt1, and histone H3 (reviewed in Hashimoto et al. 2009). Somehow Dnmt1 must displace UHRF1 from the site to allow methylation. In the coming years, it will be important to understand how these multiple binding events are coordinated and whether they are cooperative for faithful mitotic inheritance of genomic methylation patterns.

2.2 De Novo Methyltransferase Dnmt3 Family

The Dnmt3 family includes two active de novo Dnmts, Dnmt3a and Dnmt3b, and one regulatory factor, Dnmt3-Like protein (Dnmt3L; Fig. 7A) (Goll and Bestor 2005). Dnmt3a and Dnmt3b have similar domain arrangements: a variable region at the amino terminus, followed by a PWWP (Pro-Trp-Trp-Pro) domain that may be involved

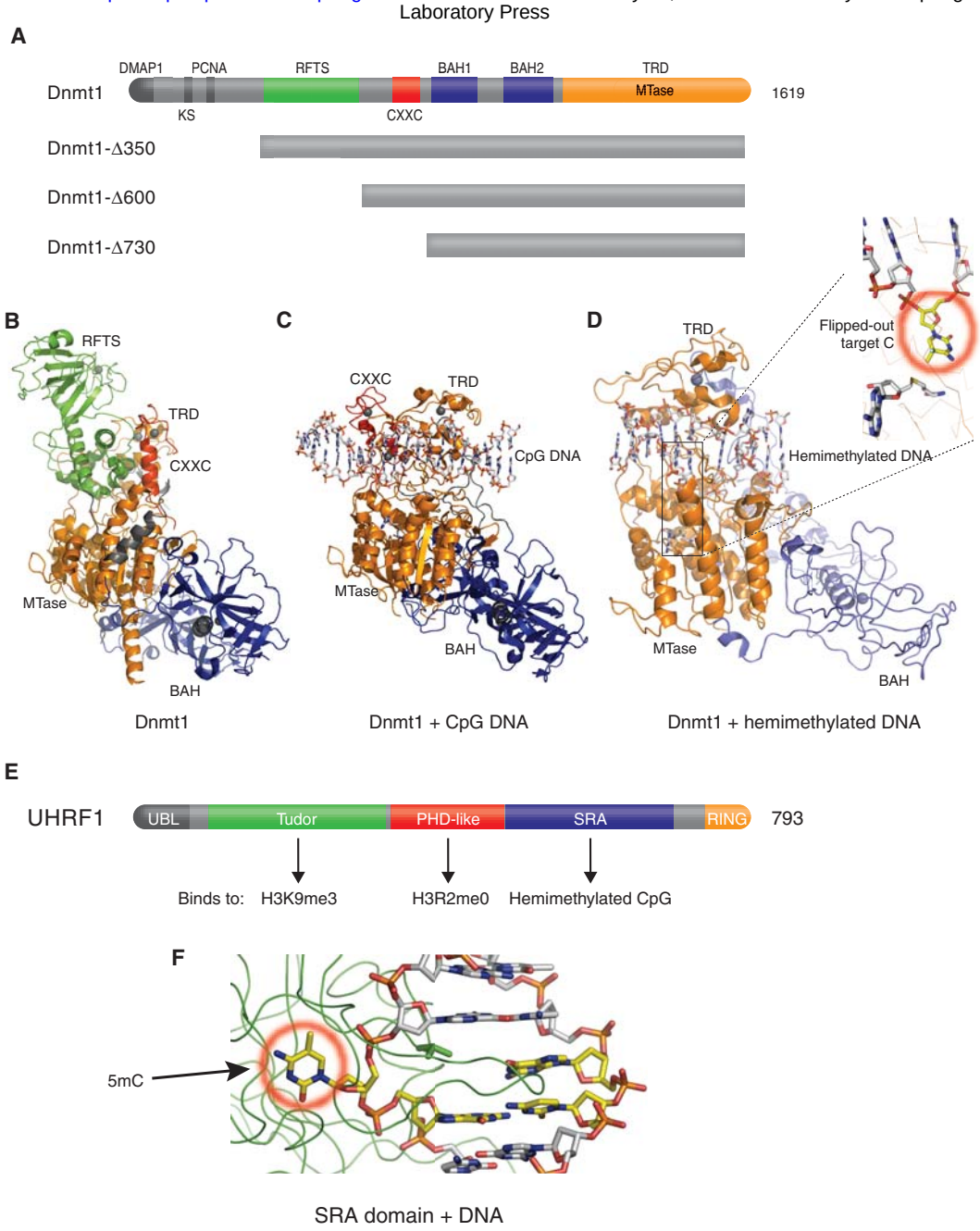

Figure 6. Structures of maintenance Dnmt1 and UHRF1. (*A*) Schematic representation of mouse Dnmt1 domain organization and available Dnmt1 amino-terminal deletion mutants. An amino-terminal region interacts with Dnmt1-associated protein(s) (DMAP1; Rountree et al. 2000). Then an adjacent lysine and serine are subject to a methylation and phosphorylation switch that determines Dnmt1 stability (Esteve et al. 2011), a PCNA (proliferating cell nuclear antigen) interacting sequence (Chuang et al. 1997), and an RFTS (Leonhardt et al. 1992) that interacts with the SET- and RING-associated (SRA) domain of UHRF1 (Achour et al. 2008). This is followed by a CpG-interacting CXXC domain (Song et al. 2011), a tandem BAH (bromo-adjacent homology) domain (Callebaut et al. 1999), and the catalytic DNA methyltransferase domain that includes the target-recognizing domain (Lauster et al. 1989) at the carboxyl terminus. (*B*) Structure of Dnmt1 in the absence of DNA (PDB 3AV4). (*C*) Structure of Dnmt1 in the presence of unmethylated CpG (PDB 3PT6). (*D*) Structure of Dnmt1 with hemimethylated CpG DNA oligonucleotides (PDB 4DA4). (*E*) UHRF1 harbors at least five recognizable functional domains: an ubiquitin-like domain at the amino terminus, followed by a tandem tudor domain recognizing H3K9me3 (Rothbart et al. 2012), a plant homeodomain (PHD) recognizing H3R2me0 (Rajakumara et al. 2011), an SRA domain recognizing hemimethylated CpG, and really interesting new gene (RING) domain at the carboxyl terminus that may endow UHRF1 with E3 ubiquitin ligase activity to histones (Citterio et al. 2004). (*F*) Structure of SRA-DNA complex illustrates 5mC flipped out from the DNA helix and bound in a cage-like pocket (circled in red; PDB 2ZO1). (Adapted from Hashimoto et al. 2009).

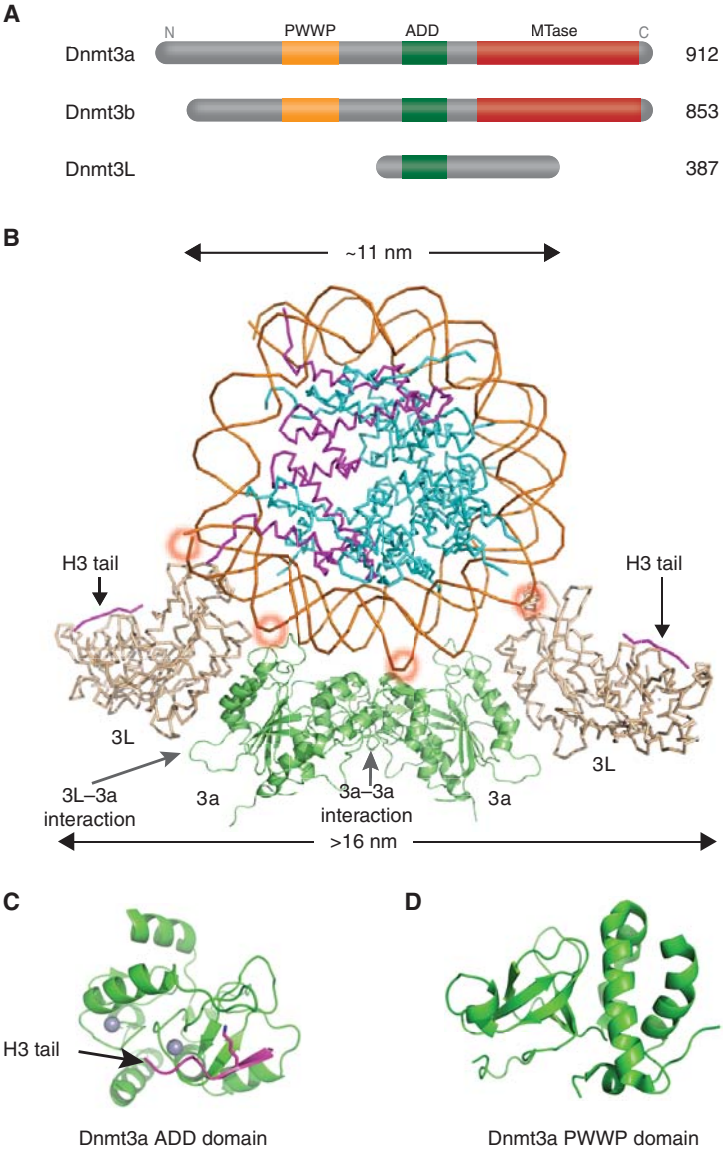

Figure 7. Structures of the de novo Dnmt3a-Dnmt3L complex. (*A*) Domain architecture of Dnmt3a, 3b, and 3L. (*B*) A nucleosome is shown docked to a Dnmt3L-3a-3a-3L tetramer (Dnmt3a is colored in green and Dnmt3L in gray; PDB 2QRV). (Adapted from Cheng and Blumenthal 2008). The position of the histone H3 amino-terminal tail (purple) bound to Dnmt3L is shown, taken from a cocrystal structure (PDB 2PVC). By wrapping the Dnmt3a/3L tetramer around the nucleosome, the two Dnmt3L molecules are able to bind both histone tails emanating from one nucleosome. The ~10 bp periodicity of binding to the major DNA groove is indicated by red circles. (*C*) Structure of the Dnmt3a ADD domain (PDB 3A1B) possibly interacting with histone tails from neighboring nucleosomes. (*D*) Structure of the Dnmt3a PWWP domain (PDB 3L1R).

in the nonspecific DNA binding for Dnmt3b (Qiu et al. 2002) or binding to histone H3K36me3 by Dnmt3a (Dhayalan et al. 2010), the Cys-rich 3-Zn-binding ADD (ATRX-DNMT3-DNMT3L) domain, and a carboxyl-terminal catalytic domain. The amino acid sequence of Dnmt3L is very similar to that of Dnmt3a and Dnmt3b in the ADD domain, but it lacks the conserved residues required for DNA methyltransferase activity in the carboxy-terminal domain. Structures are available for a complex between the carboxy-terminal domains of Dnmt3a and Dnmt3L (Jia et al. 2007) and the intact Dnmt3L in complex with a histone H3 amino-tail peptide (Fig. 7B) (Ooi et al. 2007). There is also structural data for an isolated ADD domain of Dnmt3a (Fig. 7C) (Otani et al. 2009), and isolated PWWP domains of Dnmt3a (Fig. 7D) and Dnmt3b (Qiu et al. 2002).

The phenotype of the Dnmt3L knockout mouse is indistinguishable from that of a Dnmt3a germ cell-specific conditional knockout—both have dispersed retrotransposons and aberrant germ cell patterns of de novo DNA methylation at loci that usually set parent-specific imprints (Bourc'his et al. 2001; Bourc'his and Bestor 2004; Kaneda et al. 2004; Webster et al. 2005). The interaction between Dnmt3L and Dnmt3a occurs in a minimal region located at the carboxy-terminal domain of both proteins, and is necessary for catalytic activity (Chedin et al. 2002; Suetake et al. 2004). The overall size of the Dnmt3a/Dnmt3L carboxy-terminal complex is ~16 nm long, which is greater than the diameter of an 11-nm core nucleosome (Fig. 7B). This complex contains two monomers of Dnmt3a and two of Dnmt3L, forming a tetramer with two 3L–3a interfaces and one 3a–3a interface (3L–3a–3a–3L). Substituting key, although noncatalytic residues at the Dnmt3a–3L or Dnmt3a–3a interfaces eliminates enzymatic activity, indicating that both interfaces are essential for catalysis (Jia et al. 2007).

The structure of the 3a–3a dimer interface suggests that the two active sites are located in adjacent DNA major grooves, facilitating the methylation by Dnmt3a of two CpGs separated by one helical turn, in one binding event (Fig. 7B). Methylation of CpG sites on long DNA substrates, by Dnmt3a, occurs with a periodicity of ~10 bp, suggesting a structural model in which Dnmt3a forms an oligomer docked to the DNA (Jia et al. 2007). This periodicity is also observed on maternally imprinted mouse genes (Jia et al. 2007). CpG methylation patterns on human chromosome 21 also correlate with a ~10-bp methylated CpG periodicity (Zhang et al. 2009). Interestingly, the ~10-bp methylation periodicity was evident in embryonic stem cells, however, often at non-CpG sites (which are substrates of Dnmt3a as well) that occur mostly in gene bodies as opposed to regulatory regions (Lister et al. 2009). Non-CG methylation is specific to the embryonic stem cell stage, as it disappears on induced differentiation and is restored in induced pluripotent stem cells. In the plant, *Arabidopsis thaliana* 10-bp periodic non-CpG DNA methylation by DRM2 (which is related to mammalian Dnmt3a) has similarly been observed (Cokus et al. 2008).

3 INTERPLAY BETWEEN DNA METHYLATION AND HISTONE MODIFICATION

3.1 Dnmt3L Connects Unmethylated H3K4 to De Novo DNA Methylation

Genome-scale DNA methylation profiles suggest that DNA methylation is correlated with histone methylation patterns (Meissner et al. 2008). Specifically, DNA methylation is correlated with the absence of H3K4 methylation and the presence of H3K9 methylation. Considering the inverse relationship between H3K4 methylation and DNA methylation, it is important to note that for the mammalian LSD histone demethylases, whose substrates include H3K4me2/me1, LSD1 is absolutely essential in maintaining global DNA methylation (Wang et al. 2009a) and LSD2 in establishing maternal DNA genomic imprints (Ciccone et al. 2009). Indeed, disruption of LSD1 results in earlier embryonic lethality and a more severe hypomethylation defect than disruption of the Dnmts themselves (Wang et al. 2009a).

The mammalian Dnmt3L-Dnmt3a de novo DNA methylation machinery can translate patterns of H3K4 methylation into heritable patterns of DNA methylation that mediate transcriptional silencing of the affected sequences (Ooi et al. 2007). Peptide interaction assays showed that Dnmt3L specifically interacts with the extreme amino terminus of histone H3; this interaction was strongly inhibited by H3K4 methylation, but was insensitive to modifications at other positions (Ooi et al. 2007). Cocrystallization of Dnmt3L with the amino tail of H3 showed this tail bound to the ADD domain of Dnmt3L (Fig. 7B), and substitution of key residues in the binding site eliminated the H3–Dnmt3L interaction. The main in vivo interaction partners of epitope-tagged Dnmt3L are Dnmt3a2, a shorter isoform of Dnmt3a (Chen et al. 2002), Dnmt3b, and the four core histones (Ooi et al. 2007). Given Dnmt3a and Dnmt3b bind nucleosomal DNA (Sharma et al. 2011), the data suggest that Dnmt3L is a probe of H3K4 methylation, and if the methylation is absent, then Dnmt3L induces de novo DNA methylation by docking activated Dnmt3a to the nucleosome.

Histone-Dnmt3L-Dnmt3a–DNA interactions have been studied in the budding yeast *S. cerevisiae* (Hu et al. 2009), which has no detectable DNA methylation (Proffitt et al. 1984) and lacks Dnmt orthologs. Introduction of the murine methyltransferases Dnmt1 or Dnmt3a leads to detectable, but extremely low levels of DNA methylation (Bulkowska et al. 2007). In contrast, a substantially higher level of de novo methylation can be achieved in yeast by coexpressing murine Dnmt3a and Dnmt3L (Hu et al. 2009). This induced DNA methylation was found preferentially in heterochromatic regions where H3K4 methylation is rare. When genes for components of the H3K4-methylating complex were disrupted in the context of Dnmt3a/3L overexpression, a greater level of genomic DNA methylation was observed. Deletions or targeted mutations in the ADD domain of Dnmt3L inhibited both global DNA methylation and the ability of Dnmt3L to associate with an H3K4me0 peptide. These same Dnmt3L mutants failed to restore normal DNA

methylation to a specific promoter when introduced into embryonic stem cells from Dnmt3L$^{-/-}$ mice (Hu et al. 2009).

The above data has led to a model in which Dnmt3L binds to H3K4me0 (via its ADD domain) and recruits Dnmt3a to regions of chromatin where H3K4 is unmethylated. Such a model could explain part of the puzzle of how DNA methylation patterns are established de novo during embryonic and germ cell development, windows of time in which both proteins are expressed (Kato et al. 2007). However, whereas Dnmt3a and 3b expression is retained in somatic cells, Dnmt3L is expressed poorly, if at all, in differentiated cell types. This raises the question of how de novo DNA methylation is restricted in somatic cells, whether Dnmt3a and 3b alone are capable of discriminating H3K4 methylation status, and (if so) the structural basis for that discrimination. The key probably lies in the fact that, in vitro, the ADD domains of Dnmt3a or Dnmt3b possess the same H3 tail-binding specificity as that of Dnmt3L (Zhang et al. 2010), and a structure of the Dnmt3a ADD domain in complex with an amino-terminal-tail peptide from histone H3 indicates that the ADD domain is sufficient to recognize H3K4me0 (Fig. 7C) (Otani et al. 2009). Furthermore, Jeong et al. showed that in nuclei from HCT116 human colon cancer cells (which do not express DNMT3L) almost all of the cellular DNMT3a and 3b (but not DNMT1) was associated with nucleosomes (Jeong et al. 2009). Chromatin binding of DNMT3a and 3b required an intact nucleosomal structure, although no other chromatin factors, suggesting that DNMT3a and 3b alone are capable of direct interaction (via the ADD domain) with chromatin components (H3K4me0) in addition to DNA.

3.2 MLL1 Links H3K4 Methylation to Unmethylated CpGs

In humans there are at least eight HKMTs with specificity for H3K4. These include the mixed lineage leukemia (MLL) genes, MLL1–MLL5, hSET1a, hSET1b, and ASH1. MLL1/SET1-associated methyltransferase activity appears to be functional only in the context of multiprotein complexes; characterization of these reveals distinct multiprotein complexes for each with several shared components (reviewed in Cosgrove and Patel 2010). The MLL family plays an important role in embryonic development and is necessary for methylation of H3K4 at a subset of genes in the human and mouse genomes, particularly the HOX gene clusters (Ansari and Mandal 2010). Translocations involving MLL genes are involved in the etiology of myeloid and lymphoid leukemias. Considering the inverse relationship between H3K4 methylation and DNA methylation, it is interesting to note that disruption of the MLL1 gene in mice results in loss of H3K4 methylation and de novo DNA methylation at some *Hox* gene promoters (Milne et al. 2002; Terranova et al. 2006), suggesting that MLLs, directly or indirectly (through H3K4 methylation), prevent DNA methylation or perhaps stabilize unmethylated DNA. In fact, MLL proteins contain a CXXC domain, an evolutionarily conserved domain that mediates selective binding to unmethylated CpGs (Fig. 8A). The CXXC interaction with unmethylated CpGs was confirmed by a solution structure of an MLL1-CXXC domain complexed with unmethylated DNA (Fig. 8B) (Cierpicki et al. 2010) and an X-ray structure of DNMT1 in complex with unmethylated DNA (Fig. 6C) (Song et al. 2011). Structurally, the CXXC domain has a novel fold arranged in an elongated shape (Fig. 8B). The CXXC domain binds DNA in a clamp-like manner with the long axis of the structure linking the two Zn ions nearly perpendicular to the DNA axis (Fig. 8B) (Cierpicki et al. 2010).

The Set1 H3K4 methyltransferase also appears to interact with unmethylated DNA, although in this case it is via the Cfp1 accessory protein, which contains a CXXC domain (Fig. 8A) (Lee and Skalnik 2005; Lee et al. 2007). High throughput sequencing of Cfp1-bound chromatin identified a notable concordance between H3K4me3 and Cfp1 at unmethylated CpG islands in the mouse brain (Thomson et al. 2010). Also, Cfp1 binds specifically to the unmethylated allele of loci that are allele-specifically DNA methylated (e.g., imprinted loci, Xist gene). Depletion of Cfp1 results in a marked reduction in H3K4me3 genome-wide. The targeting of Cfp1 to CpG islands is independent of promoter activity as the insertion of an untranscribed, unmethylated CpG-dense construct into the genome of embryonic stem cells is sufficient to nucleate Cfp1 binding and H3K4me3. This suggests that unmethylated CpGs recruit Cfp1 and the associated methyltransferase Set1 creates new marks of H3K4me3 on the local chromatin (illustrated in Fig. 1 of Blackledge et al. 2013).

CXXC domains are also found in Dnmt1 (Fig. 6), the methyl-CpG-binding protein MBD1 (Jorgensen et al. 2004), and Tet1, a Jumonji-like enzyme that catalyzes the conversion of 5mC to 5-hydroxymethylcytosine (Fig. 8A) (Tahiliani et al. 2009). Interestingly, the recurrent translocation, t(10;11)(q22;q23), has been described in acute myelogenous leukemias, and results in a fusion transcript that juxtaposes the first six exons of MLL1 (containing the AT hook and CXXC) to the carboxyl-terminal one third of TET1, thus "replacing" the TET1 CXXC with the MLL1 CXXC (labeled as the breakpoint in MLL1; Fig. 8A) (Ono et al. 2002; Lorsbach et al. 2003). Whether this leads to altered targeting of methyl hydroxylation remains to be determined.

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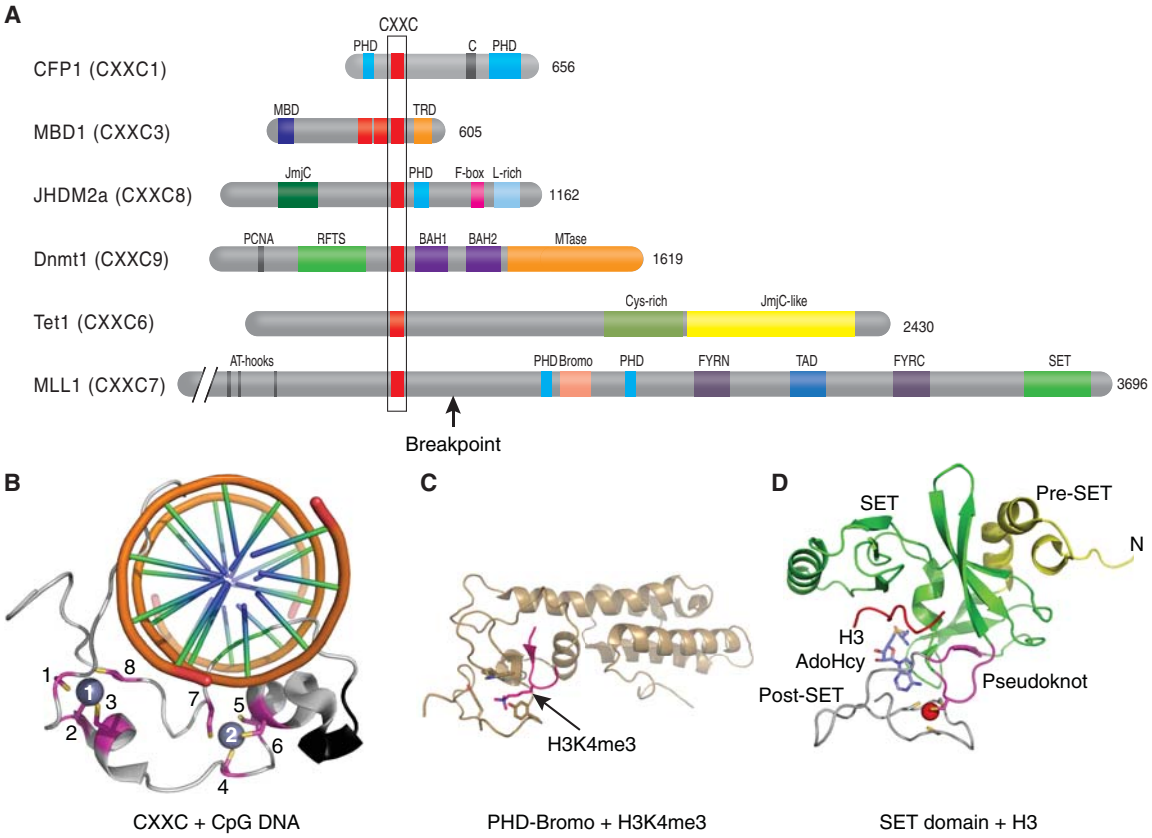

Figure 8. CpG-interacting proteins including MLL1. (*A*) Domain architecture of CXXC domain-containing proteins. (*B–D*) The structures of three isolated MLL1 domains in complex with interacting histones or DNA: (*B*) the amino-terminal CXXC domain in complex with CpG DNA (PDB 2KKF; Cierpicki et al. 2010), (*C*) the central PHD-bromodomain in complex with H3K4me3 peptide (PDB 3LQJ; Wang et al. 2010), and (*D*) the carboxyl-terminal SET domain (PDB 2W5Z; Southall et al. 2009).

3.3 JHDM1 Binds CpG DNA and Demethylates H3K36me2

Like the histone H3K4 methyltransferases of the MLL/SET1 family, the Jumonji domain-containing histone demethylases, JHDM1A (also known as CXXC8 or KDM2A; Fig. 8A) and JHDM1B (CXXC2 or KDM2B) have CXXC DNA-binding domains (Tsukada et al. 2006). Like the Set1-Cfp1 complex, JHDM1A is recruited to unmethylated CpG islands on a genome-wide scale via its CXXC domain (Blackledge et al. 2010). Like Cfp1, its localization to CpG islands is independent of promoter activity and gene-expression levels, and correlated with the selective depletion of H3K36me2/me1 within the CpG island, but not surrounding regions or the bodies of genes (see Fig. 1 of Blackledge et al. 2013); knockdown of JHDM1A/KDM2A results in the selective accumulation of H3K36me2 in these regions. Consistent with the idea that DNA methylation restricts the localization of CXXC proteins, JHDM1A/KDM2A becomes mislocalized to DNA hypomethylated pericentric heterochromatin in DNA methyltransferase Dnmt1$^{-/-}$ mice. Although in vitro studies suggest that the CXXC domains can bind a single CpG site with micromolar affinity, both the Set1-Cfp1 and JHDM1A/KDM2A studies suggest that the targeting of CXXC proteins in vivo is dependent on CpG density as well as its methylation status. It could be possible that these proteins oligomerize and form nucleoprotein filaments on CpG-dense DNA, in a manner similar to that described for the DNA methyltransferase Dnmt3a-3L complex (Jurkowska et al. 2008). To reinforce the correlation between DNA methylation and H3K36 methylation, the PWWP domain of Dnmt3a is capable of binding H3K36me3 (Dhayalan et al. 2010) and directs DNA methylation (Chen et al. 2004).

3.4 Linkage between H3K9 Methylation and DNA Methylation

Methylation at H3K9 is positively correlated with DNA methylation, in contrast to its negative correlation with

H3K4 methylation. There is in vivo evidence that the H3K9-linked DNA methylations represent an evolutionarily conserved silencing pathway. Studies in *Neurospora* and *Arabidopsis* have shown a strict dependence of DNA methylation on the H3K9 methyltransferases Dim-5 and KRYPTONITE (KYP; Tamaru and Selker 2001; Jackson et al. 2002; Tamaru et al. 2003). The SRA domain of KYP (also known as SUVH4) binds directly to methylated CHG-containing oligonucleotides (Johnson et al. 2007), whereas a plant-specific DNA CHROMOMETHYLASE3 (CMT3), responsible for CHG methylation, binds H3K9me2-containing nucleosomes via its associated BAH and chromodomains within the same polypeptide (Du et al. 2012), resulting in a self-reinforcing loop between H3K9me2 and CHG methylation in plants.

With regard to mammals, G9a, and GLP, two related euchromatin-associated H3K9 methyltransferases form heterodimers and have been implicated in DNA methylation at various loci, including imprinting centers, retrotransposons and satellite repeats, a G9a/GLP target promoter, an Oct4 promoter, and a set of embryonic genes (reviewed in Collins and Cheng 2010; Shinkai and Tachibana 2011). Furthermore, G9a is required for de novo DNA methylation and the establishment of silencing of newly integrated proviruses in murine embryonic stem cells (Leung et al. 2011). The G9a/GLP heterodimer interacts with a chromodomain protein MPP8, which in turn interacts with DNA methyltransferase Dnmt3a and/or methylated H3K9 (Kokura et al. 2010; Chang et al. 2011). Together, these findings provide a molecular explanation, at least in part, for the co-occurrence of DNA methylation and H3K9 methylation in chromatin.

The functional relationships between DNA methylation and two other H3K9 methyltransferases in mammals are complex and context dependent, as deletion of Suv39h1/h2 (Lehnertz et al. 2003) or SETDB1 (Matsui et al. 2010) has only a minor impact on DNA methylation at constitutive heterochromatin or endogenous retroelements, respectively. A particularly interesting observation is that the methyl-CpG-binding domain protein MBD1 (Fig. 8A) forms a stable complex with SETDB1 (Sarraf and Stancheva 2004; Lyst et al. 2006) as well as the Suv39h1/HP1 complex (Fujita et al. 2003), constituting the heterochromatin-specific H3K9me3 writer and reader. The methyl-CpG-binding domain (MBD) is present in a family of proteins conserved throughout the eukaryotic lineage. This domain, in some but not all cases, confers the ability to bind fully methylated CpGs. Mammals have five well-characterized members of this family, each with unique biological characteristics (reviewed in Dhasarathy and Wade 2008; Li and Zhang 2014). SETDB1 also contains an intrinsic putative MBD domain with two conserved DNA-interacting arginine residues known to make direct contact with DNA in the structures of the MBD domain (reviewed in Hashimoto et al. 2010). It remains to be seen whether the putative MBD domain of SETDB1 is similarly able to selectively bind methylated DNA. The intrinsic or associated coupling of a DNA methylation "reader" (MBD) with an H3K9me3 "writer" (SETDB1) implies an interdependent mechanism for the propagation or maintenance of these marks.

Finally, the identification of UHRF1 and its potential role in modulating the specificity of Dnmt1 for hemimethylated CpG sites and binding of histones provides another layer to the mechanism that ensures the faithful transmission of epigenetic information during DNA replication. Given that UHRF1 has the potential to interact with both hemimethylated CpGs (via the SRA domain) and H3K9me3 (via the Tudor domain), and is known to interact with a wide variety of epigenetic regulators, including Dnmt1, the H3K9 methyltransferase G9a and a histone acetyltransferase Tip60, it is possible that UHRF1 and the proteins in this larger complex play a more central role in coupling the transmission of DNA and histone methylation (H3K9, in particular) during mitotic cell division. It is interesting to know that, like plant CMT3, the mammalian Dnmt1 (and its homologs in *Neurospora* DIM2 and plant MET1) contains a BAH domain(s) within the same polypeptide (Fig. 6A). It remains to be seen whether the BAH domains of Dnmt1 are similarly able to bind methylated histones, either methylated H3K9, as recognized by the BAH domain of CMT3, or methylated H4K20, as recognized by the BAH domain of human ORC1 (origin of replication complex; Kuo et al. 2012).

3.5 PHF8 Binds H3K4me3 and Demethylates H3K9me2

The examples discussed so far provide a molecular explanation, at least in part, for the inverse correlation of DNA methylation and H3K4 methylation, as well as the co-occurrence of DNA methylation and H3K9 methylation in chromatin. The functional linkage of DNA methylation, H3K4 methylation, and H3K9 methylation is further illustrated by the finding that treatment with 5-aza-2′-deoxycytidine (5-aza), a DNA-demethylating drug (Yoo et al. 2007), leads to depletion of DNA methylation and H3K9 methylation, and a corresponding increase in H3K4 methylation (Nguyen et al. 2002). How is the inverse correlation of H3K4 and H3K9 methylation maintained?

PHF8 belongs to a small family of Jumonji proteins with three members in mice and human (PHF2, PHF8, and KIAA1718; Klose et al. 2006). Mutations in the PHF8 gene lead to X-linked mental retardation (Loenarz

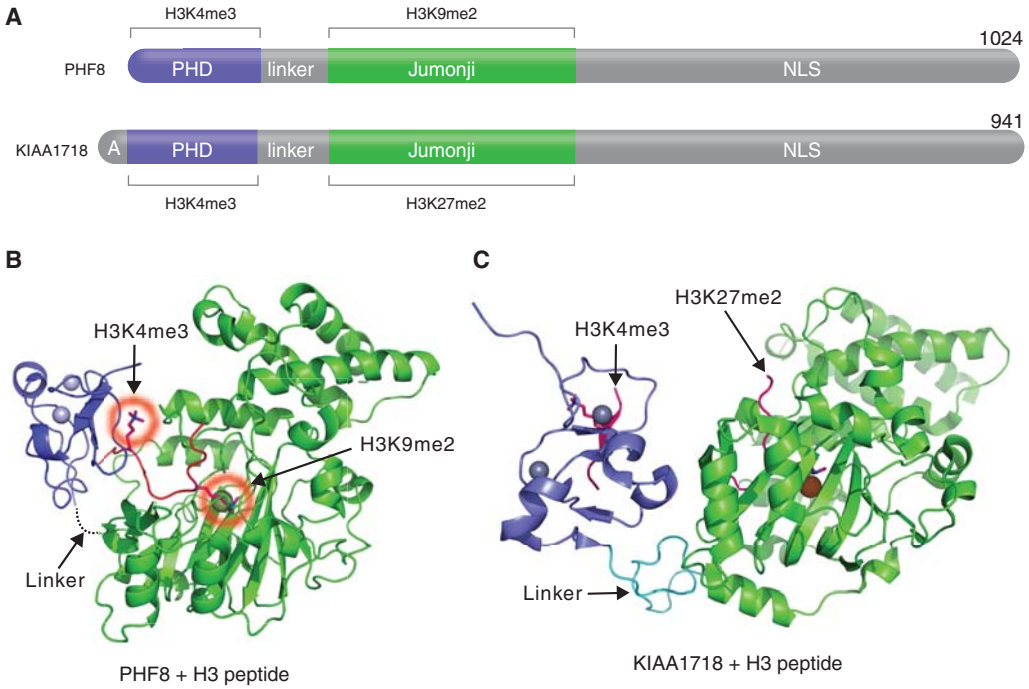

Figure 9. Coordinated methyllysine erasure between a Jumonji and a PHD. (*A*) Schematic representations of PHF8 and KIAA1718 domain structure. (*B*) A bent conformation of PHF8 bound to a histone H3 peptide (in red) containing K4me3 and K9me2 (circled in red; PDB 3KV4). (*C*) An extended conformation of KIAA1718 (PDB 3KV6). The position of the histone H3 peptide is taken from a cocrystal structure of *Caenorhabditis elegans* KIAA1718 (PDB 3N9P).

et al. 2010), and knockdown of KIAA1718 (also known as JHDM1D) and PHF8 homologs in zebrafish cause brain defects (Qi et al. 2010; Tsukada et al. 2010). These proteins harbor two domains in the amino-terminal half (Fig. 9A): a PHD domain that binds H3K4me3 and a Jumonji domain that demethylates H3K9me2, H3K27me2, or H4K20me1. However, the presence of H3K4me3 on the same peptide as H3K9me2 makes the doubly methylated peptide a significantly better substrate of PHF8 (Feng et al. 2010; Fortschegger et al. 2010; Horton et al. 2010; Kleine-Kohlbrecher et al. 2010). In contrast, the presence of H3K4me3 has the opposite effect in that it diminishes the H3K9me2 demethylase activity of KIAA1718 with no adverse effect on its H3K27me2 activity (Horton et al. 2010). Differences in substrate specificity between the two enzymes are explained by a bent conformation of PHF8 (Fig. 9B), allowing each of its domains to engage their respective targets, and an extended conformation of KIAA1718, which prevents the access to H3K9me2 by its Jumonji domain when its PHD domain engages H3K4me3 (Fig. 9C). Thus, the structural linkage between the PHD domain binding to H3K4me3 and the placement of the catalytic Jumonji domains relative to this active epigenetic mark determines which repressive marks (H3K9me2 or H3K27me2) are removed by these demethylases. Thus, the data indicate that the PHF8 and KIAA1718 Jumonji domains on their own are promiscuous enzymes; the PHD domains and linker—a determinant for the relative positioning of the two domains—are mainly responsible for substrate specificity.

Another structural study on *C. elegans* KIAA1718 suggested that the PHD and Jumonji domains might enable a *trans*-histone peptide-binding mechanism, in which the substrate peptide associated with the PHD domain and the peptide bound to the Jumonji domain could be coming from two separate histone molecules of the same nucleosome or two neighboring nucleosomes (Yang et al. 2010). The *trans*-binding mechanism is an attractive model for PHF8 and could explain the finding that PHF8 also functions in vivo as an H4K20me1 (histone H4 monomethylated at lysine 20) demethylase, whereas its PHD domain interacts with H3K4me3 in the context of nucleosome (Liu et al. 2010; Qi et al. 2010). But one has to explain why PHF8 is only active on monomethylated H4K20 (H4K20me1), whereas it is active on dimethylated H3K9 and H3K27. One possibility is that only H4K20me1 coexists with H3K4me3 in vivo.

4 SUMMARY

Combinatorial readout of multiple covalent chromatin modifications (including DNA methylation) is an explicit

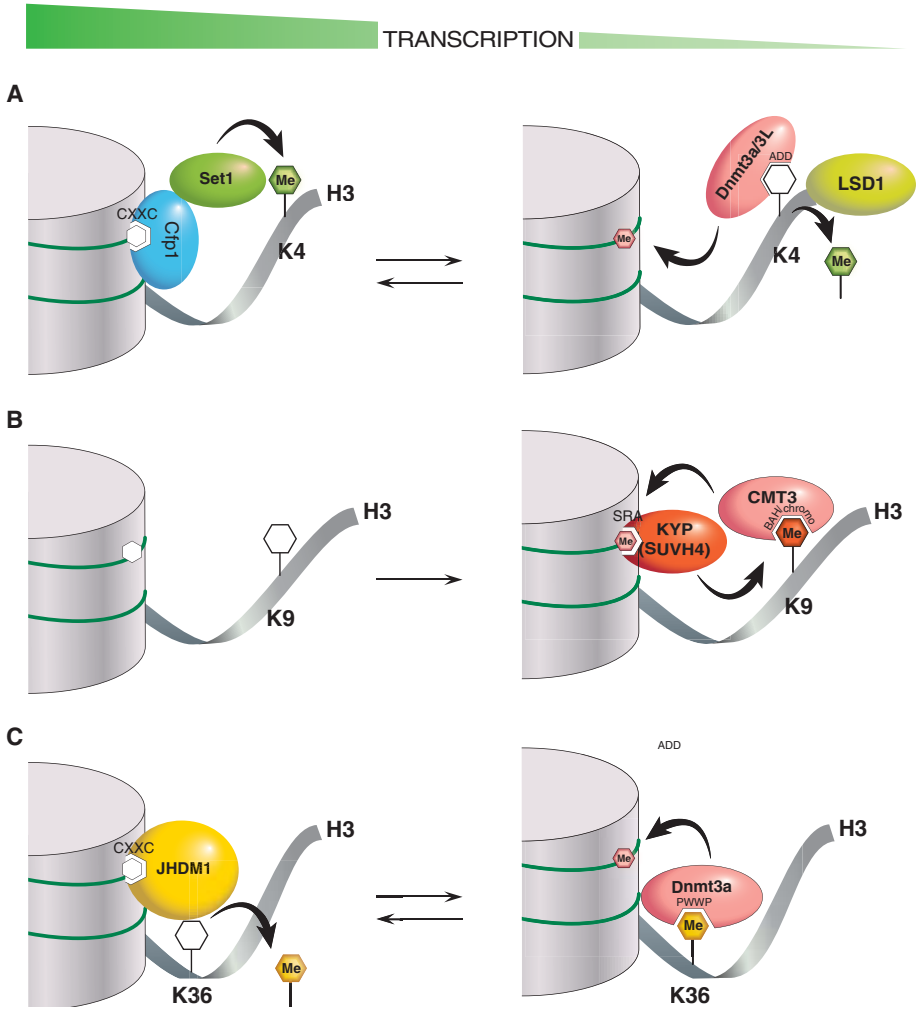

Figure 10. Cartoons of interactions that regulate DNA methylation and associated histone H3 modifications. There are three examples of PTM cross talk between histone and DNA methylation. Chromatin on the *left* represents transcriptionally active states, whereas chromatin on the *right* represents transcriptionally repressive states. The "Me"-labeled filled hexagons indicate one or more methyl groups in DNA (pink) or protein lysine residues (K). The catalytic action of methylation writers and erasers are indicated by curved black arrows. Methylation readers interact via specific labeled domains that fit in a lock-and-key fashion to methylated (filled hexagons) or unmethylated CpGs or lysines (unfilled hexagons). (*A*) Enzymatic reactions by the Dnmt3a-Dnmt3L complex and the SET1/CFP1 complex regulate the inverse correlation of DNA methylation and H3K4 methylation. (*B*) In plants, enzymatic reactions by CMT3 and KYP reinforce the correlation between DNA CHG methylation and H3K9 methylation. (*C*) Enzymatic reactions by Dnmt3a and JHDM1 positively regulate the association of DNA methylation with H3K36 methylation.

prediction of the "histone code" hypothesis (Strahl and Allis 2000; Jenuwein and Allis 2001; Turner 2007). Although it is well-accepted that DNA methylation patterns are replicated in a semiconservative fashion during cell division via a Dnmt1-dependent mechanism discussed (see also Li and Zhang 2014), one of the fundamental unresolved questions is how, and indeed whether, histone modifications are similarly "inherited." Considering that the well-studied lysine methylation events reside on histone H3 (K4, K9, K27, K36, and K79) or H4 (K20), this evokes a model in which "old" histone methylation patterns may be retained (possibly by UHRF1) and copied onto newly deposited tetramers from neighboring parental nucleosomes. Indeed, many of the SET domain histone methyltransferases contain intrinsic or associated reader domains that recognize the same mark that they generate, allowing for the copying of these marks from old to new nucleosomes. For example, G9a/GLP catalyzes H3K9me1/2 and

contains an ankyrin-repeat domain that binds H3K9me1/2 (Collins et al. 2008). Likewise, SUV39H1/2, the H3K9me3 writer, interacts with HP1, the H3K9me3 reader (reviewed in Grewal and Jia 2007). Similarly, yeast Clr4 methylates H3K9 and contains a chromodomain that binds H3K9me3 (Zhang et al. 2008). This interdomain cross talk provides a possible mechanism for propagating a methyl mark. Thus, higher organisms have evolved coordinated mechanisms of deposition and transmission of repressive chromatin marks to both DNA and histones. Enzymes that affect more complex cross talk include PHF8, which contains modules within the same polypeptide for both recognizing (PHD) and removing (Jumonji domain) two opposing methyl marks. This cross talk provides a possible mechanism for removing a repressive methyl mark (H3K9me2 or H4K20me1) based on an existing active methyl mark (H3K4me3). An even more complex situation is JARID1A, which contains multiple PHD domains for recognizing the substrate (H3K4me3) and product (H3K4me0) of its catalytic Jumonji domain, respectively (Wang et al. 2009b).

We have also discussed enzyme complexes that cross talk between DNA methylation (or lack thereof) and histone marks that are probably on the same nucleosome. These include the Dnmt3a-Dnmt3L complex, containing a reader domain for H3K4me0 coupled to DNA methyltransferase activity, whereas MLL1 (or Set1-Cfp1 complex) contains reader domains for DNA CpG and a SET domain for making methylated H3K4 (Fig. 10A). The mammalian Dnmt1-UHRF1 complex (Rothbart et al. 2012) and plant-specific CMT3 (Du et al. 2012) contain reader domains for H3K9me3/2 and DNA methyltransferase activity (Fig. 10B). The function of the Jumonji H3K36me3-specific demethylase JHDM1 is linked to the CXXC domain, which associates with unmethylated CpG DNA (Fig. 10C) (Blackledge et al. 2010).

Another intriguing observation involving DNA methylation is its mutually antagonistic relationship with histone variant H2A.Z (Zilberman et al. 2008; Conerly et al. 2010). How the exclusion is specifically established remains largely unknown. One possibility is that the histone variant H2A.Z is preferentially deposited by the remodeling ATPase complexes to regions lacking DNA methylation. Another scenario is that nucleosomes that contain the histone variant H2A.Z are no longer the substrates of DNA methyltransferases. Future experiments are needed to uncover the mechanisms of correct assembly of machinery required to accurately modify chromatin. Although the field still faces a number of critical questions, it is clear that structural analyses will continue to play a central and synergistic role, together with biochemical and genetic studies to address them.

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