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Molecular coupling of DNA methylation and histone methylation

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Abstract

The combinatorial pattern of DNA and histone modifications constitutes an epigenetic ‘code’ that shapes gene-expression patterns by enabling or restricting the transcriptional potential of genomic domains. DNA methylation is associated with histone modifications, particularly the absence of histone H3 lysine 4 methylation (H3K4me0) and the presence of H3K9 methylation. This article focuses on three protein domains (ATRX–Dnmt3–Dnmt3L [ADD], Cys–X–X–Cys [CXXC] and the methyl-CpG-binding domain [MBD]) and the functional implications of domain architecture in the mechanisms linking histone methylation and DNA methylation in mammalian cells. The DNA methyltransferase DNMT3a and its accessory protein DNMT3L contain a H3K4me0-interacting ADD domain that links the DNA methylation reaction with unmodified H3K4. The H3K4 methyltransferase MLL1 contains a CpG-interacting CXXC domain that may couple the H3K4 methylation reaction to unmethylated DNA. Another H3K4 methyltransferase, SET1, although lacking an intrinsic CXXC domain, interacts directly with an accessory protein CFP1 that contains the same domain. The H3K9 methyltransferase SETDB1 contains a putative MBD that potentially links the H3K4 methylation reaction to methylated DNA or may do so through the interaction with the MBD containing protein MBD1. Finally, we consider the domain structure of the DNA methyltransferase DNMT1, its accessory protein UHRF1 and their associated proteins, and propose a mechanism by which DNA methylation and histone methylation may be coordinately maintained through mitotic cell division, allowing for the transmission of parental DNA and for the histone methylation patterns to be copied to newly replicated chromatin.

Keywords

ADD; CpG-binding domain; CXXC; H3K4me0-binding domain; hemi-methyl-CpG-binding domain; MBD; methyl-CpG-binding domain; SRA

Epigenetics, the cell-type-specific interpretation of genetic material, relies on DNA methylation and post-translational histone modifications to regulate gene function. Nucleosomes, the fundamental building unit that packages DNA, consist of approximately 146 bp of DNA wrapped approximately 1.8-times around a histone octamer that is evolutionarily conserved [1]. Histones are subject to considerable post-translational modifications including acetylation, methylation, ubiquitylation and sumoylation of lysine

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residues and methylation of arginine residues [2]. The combinatorial pattern of DNA and histone modifications constitutes an epigenetic 'code' that shapes gene-expression patterns by enabling or restricting the transcriptional potential of genomic domains. The code is written by sequence and site-specific modification enzymes and interpreted by effector molecules that mediate the assembly of higher-order chromatin structures. Epigenetic regulation plays a fundamental role in gene expression [3], DNA replication [4] and, recombination and repair [5], and is responsible for stem cell development [6] and cellular differentiation [7]. Moreover, alterations in epigenetic modifications accompany the aging process and contribute to the pathogenesis of cancer [8–14] and degenerative diseases [15,16].

One broad theme that has emerged is that a web of interactions tightly coordinates the modification of a segment of DNA and its associated histones, particularly histone H3. This article focuses on three protein domains (ATRX–Dnmt3–Dnmt3L [ADD], Cys–X–X–Cys [CXXC] and the methyl-CpG-binding domain [MBD]) and how they characterize the functional links between histone and DNA modification in mammalian cells. In particular we consider the relationship between DNA CpG methylation and histone H3 methylation on lysines 4 and 9 [17–23]. DNA methylation and histone lysine methylation are intimately connected with one another [17,19–21]. In fact, genome-scale DNA methylation profiles suggest that DNA methylation is correlated to histone methylation patterns [18]. Specifically, DNA methylation is associated with the absence of H3K4 methylation (H3K4me0) and the presence of H3K9 methylation, but has little correlation with methylation of H3K27 [22]. *In vivo* studies support a molecular link between the mechanisms that maintain DNA methylation and H3K9 methylation. Studies in *Neurospora* and *Arabidopsis* have demonstrated a strict dependence of DNA methylation on the H3K9 methyltransferases Dim-5 and KRYPTONITE [24–26]. The relationship in mammals appears more complex, however, as deletion of the H3K9 trimethyltransferases SUV39H1/H2 [27] or SETDB1 [28] has only a minor impact on DNA methylation at constitutive heterochromatin or endogenous retroelements, respectively. That the mechanisms defining genomic patterns of DNA methylation, H3K4 methylation and H3K9 methylation are functionally linked is underscored by the finding that treatment with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine leads to depletion of DNA methylation and a loss of H3K9 methylation and a corresponding increase in H3K4 methylation [29].

ADD domains of Dnmt3L/3a/3b link DNA methylation to unmethylated H3K4

DNA methylation in mammals is coordinately established and maintained by two DNA methyltransferase families, the so-called '*de novo*' methyltransferases of the Dnmt3 family and the 'maintenance' methyltransferase Dnmt1. The Dnmt3 family includes two active *de novo* Dnmts, Dnmt3a and -3b, and one regulatory factor, Dnmt3L (Figure 1A). Dnmt3a and -3b have similar domain arrangements: both contain a variable region at the N-terminus, followed by a Pro–Trp–Trp–Pro domain, an ADD domain and a C-terminal catalytic domain. The amino acid sequence of Dnmt3L is very similar to that of Dnmt3a and -3b in the ADD domain (Figure 1B). Dnmt3L associates *in vivo* with not only Dnmt3b and -3a2 (a shorter isoform of Dnmt3a) [30], but also with the four core histones [31]. Peptide interaction assays and cocrystallization of Dnmt3L with the amino tail of H3 demonstrated that Dnmt3L specifically interacts with the amino terminus of histone H3 only when H3K4 is not modified [31]. These data suggest that Dnmt3L acts as a sensor for H3K4 methylation; when methylation is absent, Dnmt3L induces *de novo* DNA methylation by docking Dnmt3a to the nucleosome (Figure 1C).

Histone–Dnmt3L–Dnmt3a–DNA interactions have recently been studied in the budding yeast *Saccharomyces cerevisiae* [32], which has no detectable DNA methylation [33] and

lacks Dnmt orthologs. Introduction of the murine methyltransferase Dnmt1 or -3a in yeast leads to detectable, but extremely low levels of DNA methylation [34]. By contrast, a substantially higher level of *de novo* methylation could be achieved in yeast by coexpressing murine Dnmt3a and -3L [32]. This induced DNA methylation was found preferentially in heterochromatic regions where H3K4 methylation is rare. When genes for components of the H3K4-methylating complex COMPASS/Set1 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 (Figure 1B) inhibited both global DNA methylation and the ability of Dnmt3L to associate with a 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 [32]. That H3K4 methylation status plays an important role in the establishment of DNA methylation is further supported by the finding that mammalian LSD1 and LSD2, two related lysine-specific demethylases whose substrates include mono- and di-methylated H3K4 (H3K4me1/2), are absolutely essential in maintaining global DNA methylation [35], or establishing maternal DNA genomic imprints [36], respectively. Indeed, disruption of LSD1 results in earlier embryonic lethality and a more severe hypomethylation defect than disruption of the Dnmts themselves [35].

The aforementioned data lead 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, when both proteins are expressed [37]. 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. To this end, recent work by Jeung *et al.* demonstrated 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 [38]. Chromatin binding of DNMT3a and -3b required an intact nucleosomal structure, though no other chromatin factors (e.g., HP1 and MBD proteins), suggesting that DNMT3a and -3b alone are capable of direct interaction with chromatin components in addition to DNA. Furthermore, recent *in vitro* studies indicate that the ADD domains of Dnmt3a or -3b possess the same H3 tail binding specificity as that of Dnmt3L [39]. Indeed, 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 (Figure 1D) [40].

The ADD domain consists of 12 cysteines, with the first four forming a 4-Cys zinc finger, and the last eight forming a plant homeodomain (PHD) finger that adopts a ‘cross-braced’ topology of the binding of two Zn atoms (Figure 1B) [41]. The two fingers pack together to form a single globular domain. The two-aspartate motif (DxD) that mediates recognition of H3K4me0 in the structurally characterized Dnmt3a and -3L are located between the two fingers, that is, between the fourth and fifth cysteine (Figure 1B). Interestingly, UHRF1 (ubiquitin-like, containing PHD and really interesting new gene [RING] finger domains 1), a modular multidomain protein important in regulating DNMT1-mediated replication-coupled DNA methlyation [42,43] (reviewed in [44,45]), contains a similar 12-cys architecture (Figure 1B), consisting of a 4-Cys zinc finger and a PHD finger, as illustrated by an NMR structure for the ADD-like domain of human UHRF2 (Protein Data Bank ID 2E6S). The major difference is that the 4-Cys zinc finger and the PHD finger are only one residue apart, and thus lack the H3K4me0-interacting acidic residue(s) between the fourth and fifth cysteine (Figure 1B). Although the UHRF1 ADD-like domain is known to bind the histone H3 tail [46,47], it remains to be seen whether it also senses the methylation state of H3K4.

Another known H3K4me0 binder is the PHD domain of BHC80 [48]. BHC80 (also known as PHF21A) is a component of the LSD1 complex [49]. Like the ADD domain of the Dnmt3 family, the PHD finger of BHC80 binds unmethylated H3K4 and this interaction is specifically abrogated by methylation at this site. Although BHC80 has a conserved DxD motif in its corresponding position (Figure 1B), the PHD domain alone (without the DxD motif) is sufficient to bind H3K4me0 [48]. Asp489 of BHC80, which is located before cysteine 5 but is not part of the DxD motif (Figure 1B), forms an electrostatic interaction with H3K4 [48]. Similarly, the first of two PHD fingers in the autoimmune regulator (AIRE), which also have an aspartate residue in the corresponding position (Asp297), bind to histone H3 tails unmethylated at K4 [50,51].

Another known H3K4me0-specific interaction occurs within the nucleosome remodeling histone deacetylase (NuRD) complex, a multisubunit enzyme complex with roles in both transcriptional regulation and in replication-coupled chromatin assembly [52]. This complex possesses two distinct enzymatic activities that affect chromatin structure and function, an ATP-dependent chromatin remodeling activity (CHD3/4) and histone deacetylase activity (HDAC1/2). The NuRD complex has been demonstrated to bind to histone H3 amino terminal peptides, and this interaction is abrogated by methylation of H3K4 [53,54]. Recently, the NuRD–H3K4me0 interaction has been mapped to CHD4 [55]. CHD4 contains two PHD fingers, the second of which (PHD-2) recognizes the N-terminal tail of histone H3 in an interaction that is inhibited by the methylation of H3K4. Sequence alignment indicates that the first and second PHD fingers of CHD4, as well as that of CHD3, are highly conserved and that the corresponding acidic residues known to be important for H3K4me0 binding in the ADD domain – those N-terminal to cysteine 5 – are also found in the CHD4 PHD-2 (ExD), but not in PHD-1 (Figure 1B). The NuRD complex also contains two members of the MBD protein family, MBD2 and MBD3 (see later). Given that H3K4me0 and methylated CpGs are typically found at sites of repressive transcription, one can speculate that the recognition of these features (H3K4me0 and methyl-CpG) allows for the recruitment and assembly of the NuRD repressor complex at these sites.

CXXC domain links H3K4 methylation to unmethylated CpGs

In humans there are at least eight histone lysine methyltransferases with specificity for H3K4. These include the *MLL* genes, *MLL1–5*, *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 [56]. The *MLL* family plays an important role in embryonic development and is necessary for the methylation of H3K4 at a subset of genes in the human and mouse genomes, particularly the *Hox* gene clusters [57]. Translocations involving *MLL* genes are involved in the etiology of myeloid and lymphoid leukemias. Considering the aforementioned inverse relationship between H3K4 methylation and DNA methylation, it is interesting to note that disruption of the *MLL1* gene in mice results in a loss of H3K4 methylation and *de novo* DNA methylation at some *Hox* gene promoters [58,59], suggesting that *MLL* genes 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 (Figure 2A) [60–62]. This interaction has now been confirmed by a solution structure of an MLL1-CXXC domain complexed with unmethylated DNA (Figure 2B) [63].

The Set1 H3K4 methyltransferases also appear to interact with unmethylated DNA, although in this case it is via an accessory protein, as was the case for Dnmt3a/Dnmt3L discussed earlier. Set1 lacks an intrinsic CXXC domain, but interacts directly with a protein

that contains the same domain, Cfp1 [64,65]. High-throughput sequencing of Cfp1-bound chromatin identified a notable concordance between H3K4me3 and Cfp1 at unmethylated CpG islands in the mouse brain [66]. At loci that exhibit allele-specific DNA methylation (e.g., imprinted loci and the *Xist* gene), Cfp1 bound specifically to the unmethylated allele. Depletion of Cfp1 resulted in a marked genome-wide reduction in H3K4me3. The targeting of Cfp1 to CpG islands was independent of promoter activity, as the insertion of an unmethylated CpG-dense construct into the genome of embryonic stem cells was 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.

Like the histone H3K4 methyltransferases of the MLL/SET1 family, the Jumonji-domain containing histone demethylase JHDM1a (also known as CXXC8 or KDM2A) also has a CXXC domain (Figure 2A) [67]. Recent work indicates that, like Cfp1, JHDM1 is recruited to unmethylated CpG islands on a genome-wide scale, and that this is dependent on the CXXC domain [68]. The localization to CpG islands was independent of promoter activity and gene-expression levels, and correlated with the selective depletion of H3K36me1/2 within the CpG island but not surrounding regions or the bodies of genes; knockdown of KDM2A resulted in the selective accumulation of H3K36me2 in these regions. Consistent with a role for DNA methylation in restricting the localization of CXXC proteins, KDM2A was mislocalized to pericentric heterochromatin in *Dnmt1*$^{-/-}$ mice. Furthermore, the lack of methylation alone does not appear to be sufficient for KDM2A recruitment *in vivo* as KDM2A does not localize to non-CpG island promoters that lack methylation. Although *in vitro* studies suggest that CXXC domains can bind a single CpG site with micromolar affinity, both the Cfp1 and KDM2A studies suggest that the targeting of CXXC proteins *in vivo* is dependent on CpG density as well as 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 Dnmt3a and -3L [69].

CXXC domains are also found in DNMT1, which is the enzyme responsible for generating 5-methyl-cytosine (5mC) [70], the MBD protein MBD1 (which binds 5mC) [71] and TET1, a Jumonji-like 2-oxoglutarate- and Fe(II)-dependent enzyme that catalyzes conversion of 5mC to 5-hydroxymethylcytosine (Figure 2A) [72]. Interestingly, a recurrent (q22;q23) translocation [10,11] has been described in acute myelogenous leukemias, it results in a fusion transcript that juxtaposes the first six exons of MLL (containing an AT hook and CXXC) to the C-terminal third of TET1, thus ‘replacing’ the TET1 CXXC with the MLL CXXC [73,74]. Whether this leads to altered targeting of methyl hydroxylation remains to be determined.

Structurally, the CXXC domain has a novel fold in which two Zn ions are each coordinated tetrahedrally by four conserved cysteines; three from one CXXCXXC motif and one from a distal cysteine residue, resulting in an elongated shape (Figure 2B) [62]. 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 (Figure 2B) [63]. The CpG-specific side-chain interactions involve a conserved (K/R/H)Q dipeptide (Figure 2B), except for the first and second CXXC domains of MBD1, which lack CpG-dependent DNA-binding activity [71]. A sequence alignment suggests that CXXC domains can be divided into two subfamilies, one with a longer DNA-binding loop containing a Lys–Phe–Gly–Gly motif, and the other, which is a shorter DNA-binding loop between cysteine 6 and cysteine 7 (Figure 2B). In the NMR structure of the MLL1 CXXC domain in complex with DNA [63], the Lys–Phe–Gly–Gly motif is located away from the DNA-binding surface (Figure 2B), suggesting a non-DNA-interacting function for this subfamily, which includes CFP1, JHDM1, DNMT1 and the third CXXC domain of MBD1.

Role for MBDs in the linkage between histone modification & DNA methylation

The 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 methylated CpGs. Mammals have five well-characterized members of this family, each with unique biological characteristics (Figure 3A) [75]. Of particular interest are recent reports indicating that in zebrafish, MBD4 (containing both a N-terminal MBD and a C-terminal thymine glycosylase domain [76]) and the cytidine deaminase AID cooperate to demethylate DNA [77]. Consistent with a role in DNA demethylation, erasure of DNA methylation in primordial germ cells is less efficient in AID-deficient animals [78], and AID is required to demethylate pluripotency genes during reprogramming of the somatic genome in embryonic stem cell fusions [79]. It is noteworthy that phosphorylation of MBD4 enhances DNA demethylation [80].

A particularly interesting observation, for the purpose of this article, is the fact that MBD1 forms a stable complex with SETDB1 [81,82], a H3K9 trimethylation (H3K9me3) methyltransferase responsible for the silencing of endogenous retroviruses [28,83,84], as well as the SUV39H1/HP1 complex [85], a heterochromatin-specific H3K9me3 writer and reader. SETDB1 also contains an intrinsic putative MBD domain with two conserved DNA-interacting arginine residues (Figure 3B) known to make direct contact with DNA in the structures of the MBD domain from MBD1 [86] and MeCP2 [87]. 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’ with H3K9me3 ‘writers’ implies an interdependent mechanism for the propagation or maintenance of these marks. However, whereas DNA methylation is dependent on H3K9me3 in some organisms (the *Arabidopsis* and *Neurospora* examples noted earlier), it is independent of DNA methylation in others (mammals). Indeed, as noted above, mouse cells deficient in the SUV39H1 or SETDB1 H3K9 trimethyltransferases have relatively minor defects in DNA methylation, at least at constitutive heterochromatin or endogenous retroelements [27,28]. Likewise, although genetic or pharmacologic ablation of DNA methylation in mouse or human cells leads to a concomitant depletion of H3K9 dimethylation, it has little impact on H3K9me3 [27,88,89]. Thus, the functional relationships between DNA methylation and H3K9 methylation are complex, context-dependent and are likely to be further complicated by differences in the way that trimethylation versus dimethylation at H3K9 are initiated and maintained (the latter being catalyzed by G9a and G9a-like histone methyltransferases; reviewed in [90]).

As MBD1 contains two DNA-binding domains – an MBD domain (Figure 3) that recognizes methylated CpG, and a CXXC domain (Figure 2) that binds unmethylated CpG – it is unclear whether these two domains function independently to facilitate the recruitment of MBD1 to repressive complexes or active complexes, or if they cooperate in some way. Recently, Clouaire *et al.* found that the MBD domain of MBD1 binds more efficiently to methylated DNA within a specific-sequence context and that a functional MBD domain is necessary and sufficient for recruitment of MBD1 to these loci, while DNA binding by the CXXC domain is largely dispensable [91].

UHRF1 links hemi-methylated CpGs to histone modifications during replication

Hemi-methylated CpG sites, where only one DNA strand is methylated, are transiently generated during semi-conservative DNA replication. Methylation patterns are faithfully

preserved with somatic cell division by a maintenance methylation reaction, involving both Dnmt1 and its accessory protein, UHRF1 [42,43], which maintains the fidelity of DNA methylation by recognizing these sites and selectively modifying the daughter strand CpG.

UHRF1 harbors five recognizable functional domains (Figure 1B): a ubiquitin-like domain at the N-terminus, followed by a tandem Tudor domain that binds H3K9me2/3 [92,93], an ADD-like domain that binds the histone H3 tail [46,47], a SET- and RING-associated (SRA) domain that binds hemi-methylated CpG-containing DNA [42,43,94–96] and a RING domain at the C-terminus that may endow UHRF1 with E3 ubiquitin ligase activity to histones [46]. As discussed earlier, Dnmt1 contains a CXXC domain (Figure 2) that binds unmethylated CpGs [70]. The interaction with UHRF1 provides a SRA domain that recognizes hemi-methylated CpGs, the substrate of Dnmt1. It is tempting to speculate that these two DNA-binding domains function synergistically to maintain the fidelity of DNA methylation during replication by preventing Dnmt1 from modifying unmethylated CpGs (through the masking of such sites by the CXXC domain) and directing methylation to newly synthesized hemi-methylated CpG sites (via the SRA domain). Indeed, molecular modeling suggests that the Dnmt1 catalytic domain and UHRF1 SRA domain are very unlikely to bind simultaneously to the same hemi-methylated CpG site [95]. Somehow the SRA domain must be displaced from the site to allow methylation by Dnmt1 (Figure 4).

We therefore suggest that the SRA–DNA interaction (through recognition of and flipping of the parent strand 5mC out of the helix [94–96]) serves as an anchor to keep UHRF1 at the hemi-methylated CpG site where it then recruits Dnmt1 to methylate the opposing CpG in the daughter strand. The displacement of UHRF1 from the Dnmt1-substrate site is very similar to recently discovered components of the nucleotide excision repair pathway. Alkyltransferase-like proteins (ATL) share amino acid sequence homology with O_6-alkylguanine DNA alkyltransferases, but lack the active-site cysteine and alkyltransferase activity. Structures of *Schizosaccharomyces pombe* ATL bound to damaged DNA containing O_6-methylguanine or cigarette-smoke-derived oxobutylguanine demonstrate that the damaged nucleotide is flipped out of the DNA helix into a binding pocket [97]. However, the binding of ATL to the damaged strand inhibits repair by O_6-alkylguaninetransferase *in vitro*. Thus, ATL binding to the alkylated DNA base generates a stable complex that recruits the nucleotide excision repair enzymatic protein(s), which must have the ability to displace ATL from the lesion [97].

The identification of UHRF1 and its potential role in modulating the specificity of Dnmt1 for hemi-methylated CpG sites provides another layer to the mechanisms ensuring the faithful transmission of epigenetic information during DNA replication. Given that UHRF1 has the potential to interact with both hemi-methylated CpGs (via the SRA domain) and H3K9me2/3 (via the Tudor domain), and is known to interact with a wide variety of epigenetic regulators, including Dnmt1 [42,43], the H3K9 di- and mono-methyltransferase G9a [98] and a histone acetyltransferase Tip60 [99], 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.

Future perspective

While it is well accepted that DNA methylation patterns are replicated in a semi-conservative fashion during cell division via the mechanisms discussed earlier, one of the fundamental unresolved questions is how, and indeed whether, histone modifications are similarly ‘inherited’. Recent work has shed light on this question. Through a combination of kinetic isotope labeling and mass spectroscopy studies, Xu *et al.* showed that histone H3.1–H4 tetramers remain intact and are partitioned evenly during DNA replication-dependent

chromatin assembly [100]. 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 histone methylation patterns may be 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 [101]. Likewise, SUV39H1/2, the H3K9me3 writer interacts with HP1, the H3K9me3 reader [102]. Similarly, yeast Clr4 methylates H3K9 and contains a chromodomain that binds H3K9me3 [103]. Thus, higher organisms have evolved coordinated mechanisms of deposition and transmission of repressive chromatin marks to both DNA and histones.

In summary, epigenetic modifiers are often modular, containing distinct catalytic and regulatory domains. Many act as components of large complexes such that a complete understanding of their function will require the analysis of modular catalytic activity within the context of histones, DNA and protein partners. The web of interactions detailed in this article will likely benefit from a systems approach, such as those applied to signaling networks [104].

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Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Clapier CR, Chakravarthy S, Petosa C, Fernandez-Tornero C, Luger K, Muller CW. Structure of the *Drosophila* nucleosome core particle highlights evolutionary constraints on the H2A-H2B histone dimer. Proteins 2008;71:1–7. [PubMed: 17957772]
2. Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403:41–45. [PubMed: 10638745]
3. Margueron R, Reinberg D. Chromatin structure and the inheritance of epigenetic information. Nat Rev Genet 2010;11:285–296. [PubMed: 20300089]
4. Probst AV, Dunleavy E, Almouzni G. Epigenetic inheritance during the cell cycle. Nat Rev Mol Cell Biol 2009;10:192–206. [PubMed: 19234478]
5. Barreto G, Schäfer A, Marhold J, et al. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 2007;445:671–675. [PubMed: 17268471]
6. Yuan P, Han J, Guo G, et al. Eset partners with Oct4 to restrict extraembryonic trophoblast lineage potential in embryonic stem cells. Genes Dev 2009;23:2507–2520. [PubMed: 19884257]
7. Schaefer CB, Ooi SK, Bestor TH, Bourc'his D. Epigenetic decisions in mammalian germ cells. Science 2007;316:398–399. [PubMed: 17446388]
8. Ehrlich M. DNA methylation and cancer-associated genetic instability. Adv Exp Med Biol 2005;570:363–392. [PubMed: 18727508]
9. Gronbaek K, Hother C, Jones PA. Epigenetic changes in cancer. APMIS 2007;115:1039–1059. [PubMed: 18042143]

10. Kurkjian C, Kummar S, Murgo AJ. DNA methylation: its role in cancer development and therapy. Curr Probl Cancer 2008;32:187–235. [PubMed: 18926282]
11. Gal-Yam EN, Saito Y, Egger G, Jones PA. Cancer epigenetics: modifications, screening, and therapy. Annu Rev Med 2008;59:267–280. [PubMed: 17937590]
12. Dobrovic A, Kristensen LS. DNA methylation, epimutations and cancer predisposition. Int J Biochem Cell Biol 2009;41:34–39. [PubMed: 18835361]
13. McCabe MT, Brandes JC, Vertino PM. Cancer DNA methylation: molecular mechanisms and clinical implications. Clin Cancer Res 2009;15:3927–3937. [PubMed: 19509173]
14. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2010;31:27–36. [PubMed: 19752007]
15. Urdinguio RG, Sanchez-Mut JV, Esteller M. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. Lancet Neurol 2009;8:1056–1072. [PubMed: 19833297]
16. Sananbenesi F, Fischer A. The epigenetic bottleneck of neurodegenerative and psychiatric diseases. Biol Chem 2009;390:1145–1153. [PubMed: 19747081]
17. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell 2007;128:669–681. [PubMed: 17320505]
18. Meissner A, Mikkelsen TS, Gu H, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature 2008;454:766–770. [PubMed: 18600261] ▪▪One of the first studies to describe genome-scale DNA methylation patterns at base pair resolution and to compare DNA methylation and histone methylation patterns as they change during cellular differentiation.
19. Fan S, Zhang MQ, Zhang X. Histone methylation marks play important roles in predicting the methylation status of CpG islands. Biochem Biophys Res Commun 2008;374:559–564. [PubMed: 18656446]
20. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 2009;10:295–304. [PubMed: 19308066]
21. Dindot SV, Person R, Strivens M, Garcia R, Beaudet AL. Epigenetic profiling at mouse imprinted gene clusters reveals novel epigenetic and genetic features at differentially methylated regions. Genome Res 2009;19:1374–1383. [PubMed: 19542493]
22. Laurent L, Wong E, Li G, et al. Dynamic changes in the human methylome during differentiation. Genome Res 2010;20:320–331. [PubMed: 20133333]
23. Cheng X, Blumenthal RM. Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. Biochemistry 2010;49:2999–3008. [PubMed: 20210320]
24. Tamaru H, Selker EU. A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. Nature 2001;414:277–283. [PubMed: 11713521]
25. Tamaru H, Zhang X, Debra McMillen, et al. Trimethylated lysine 9 of histone H3 is a mark for DNA methylation in *Neurospora crassa*. Nat Genet 2003;34:75–79. [PubMed: 12679815]
26. Jackson JP, Lindroth AM, Cao X, Jacobsen SE. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature 2002;416:556–560. [PubMed: 11898023]
27. Lehnertz B, Ueda Y, Derijck AA, et al. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 2003;13:1192–1200. [PubMed: 12867029]
28. Matsui T, Leung D, Miyashita H, et al. Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature 2010;464:927–931. [PubMed: 20164836] ▪▪Suggests a DNA methylation-independent H3K9 methylation pathway involving ESET (also known as SETDB1).
29. Nguyen CT, Weisenberger DJ, Velicescu M, et al. Histone H3-lysine 9 methylation is associated with aberrant gene silencing in cancer cells and is rapidly reversed by 5-aza-2′-deoxycytidine. Cancer Res 2002;62:6456–6461. [PubMed: 12438235]
30. Chen T, Ueda Y, Xie S, Li E. A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active *de novo* methylation. J Biol Chem 2002;277:38746–38754. [PubMed: 12138111]
31. Ooi SK, Qiu C, Bernstein E, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature 2007;448:714–717. [PubMed: 17687327] ▪▪Suggests a model

whereby absence of H3K4 methylation acts as a signal to recruit DNMT3L and induce DNA methylation by DNMT3a.

32. Hu JL, Zhou BO, Zhang RR, Zhang KL, Zhou JQ, Xu GL. The N-terminus of histone H3 is required for *de novo* DNA methylation in chromatin. Proc Natl Acad Sci USA 2009;106:22187–22192. [PubMed: 20018712] ▪▪Discusses how the authors tested histone–Dnmt3L–Dnmt3a–DNA interactions in the budding yeast *Saccharomyces cerevisiae*, and found that induced DNA methylation in yeast preferentially occurs in heterochromatic regions where H3K4 methylation is rare.
33. Proffitt JH, Davie JR, Swinton D, Hattman S. 5-methylcytosine is not detectable in *Saccharomyces cerevisiae* DNA. Mol Cell Biol 1984;4:985–988. [PubMed: 6374428]
34. Bulkowska U, Ishikawa T, Kurlandzka A, Trzcinska-Danielewicz J, Derlacz R, Fronk J. Expression of murine DNA methyltransferases Dnmt1 and Dnmt3a in the yeast *Saccharomyces cerevisiae*. Yeast 2007;24:871–882. [PubMed: 17640084]
35. Wang J, Hevi S, Kurash JK, et al. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet 2009;41:125–129. [PubMed: 19098913] ▪Along with [36], demonstrates that LSD1 and LSD2, two related mammalian H3K4me1/2 demethylases, are essential in maintaining global DNA methylation or establishing maternal DNA genomic imprints, respectively.
36. Ciccone DN, Su H, Hevi S, et al. KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. Nature 2009;461:415–418. [PubMed: 19727073] ▪Along with [35], demonstrates that LSD1 and LSD2, two related mammalian H3K4me1/2 demethylases, are essential in maintaining global DNA methylation or establishing maternal DNA genomic imprints, respectively.
37. Kato Y, Kaneda M, Hata K, et al. Role of the Dnmt3 family in *de novo* methylation of imprinted and repetitive sequences during male germ cell development in the mouse. Hum Mol Genet 2007;16:2272–2280. [PubMed: 17616512]
38. Jeong S, Liang G, Sharma S, et al. Selective anchoring of DNA methyltransferases 3A and 3B to nucleosomes containing methylated DNA. Mol Cell Biol 2009;29:5366–5376. [PubMed: 19620278] ▪Revealed that almost all of the cellular DNMT3a and -3b was associated with nucleosomes, primarily associated with methylated short interspersed repetitive nuclear elements and long interspersed repetitive nuclear elements as well as some single-copy methylated CpG islands.
39. Zhang Y, Jurkowska R, Soeroes S, et al. Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. Nucleic Acids Res 2010;38(13):4246–4253. [PubMed: 20223770]
40. Otani J, Nankumo T, Arita K, Inamoto S, Ariyoshi M, Shirakawa M. Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. EMBO Rep 2009;10:1235–1241. [PubMed: 19834512]
41. Argentaro A, Yang JC, Chapman L, et al. Structural consequences of disease-causing mutations in the ATRX-DNMT3-DNMT3L (ADD) domain of the chromatin-associated protein ATRX. Proc Natl Acad Sci USA 2007;104:11939–11944. [PubMed: 17609377]
42. Bostick M, Kim JK, Esteve PO, Clark A, Pradhan S, Jacobsen SE. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. Science 2007;317:1760–1764. [PubMed: 17673620]
43. Sharif J, Muto M, Takebayashi S, et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature 2007;450:908–912. [PubMed: 17994007]
44. Hashimoto H, Horton JR, Zhang X, Cheng X. UHRF1, a modular multi-domain protein, regulates replication-coupled crosstalk between DNA methylation and histone modifications. Epigenetics 2009;4:8–14. [PubMed: 19077538]
45. Unoki M, Brunet J, Mousli M. Drug discovery targeting epigenetic codes: the great potential of UHRF1, which links DNA methylation and histone modifications, as a drug target in cancers and toxoplasmosis. Biochem Pharmacol 2009;78:1279–1288. [PubMed: 19501055]
46. Citterio E, Papait R, Nicassio F, et al. Np95 is a histone-binding protein endowed with ubiquitin ligase activity. Mol Cell Biol 2004;24:2526–2535. [PubMed: 14993289]

47. Karagianni P, Amazit L, Qin J, Wong J. ICBP90, a novel methyl K9 H3 binding protein linking protein ubiquitination with heterochromatin formation. Mol Cell Biol 2008;28:705–717. [PubMed: 17967883]

48. Lan F, Collins RE, De Cegli R, et al. Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression. Nature 2007;448:718–722. [PubMed: 17687328]

49. Iwase S, Januma A, Miyamoto K, et al. Characterization of BHC80 in BRAF–HDAC complex, involved in neuron-specific gene repression. Biochem Biophys Res Commun 2004;322:601–608. [PubMed: 15325272]

50. Org T, Chignola F, Hetényi C, et al. The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. EMBO Rep 2008;9:370–376. [PubMed: 18292755]

51. Chignola F, Gaetani M, Rebane A, et al. The solution structure of the first PHD finger of autoimmune regulator in complex with non-modified histone H3 tail reveals the antagonistic role of H3R2 methylation. Nucleic Acids Res 2009;37:2951–2961. [PubMed: 19293276]

52. Helbling Chadwick L, Chadwick BP, Jaye DL, Wade PA. The Mi-2/NuRD complex associates with pericentromeric heterochromatin during S phase in rapidly proliferating lymphoid cells. Chromosoma 2009;118:445–457. [PubMed: 19296121]

53. Zegerman P, Canas B, Pappin D, Kouzarides T. Histone H3 lysine 4 methylation disrupts binding of nucleosome remodeling and deacetylase (NuRD) repressor complex. J Biol Chem 2002;277:11621–11624. [PubMed: 11850414]

54. Nishioka K, Chuikov S, Sarma K, et al. Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. Genes Dev 2002;16:479–489. [PubMed: 11850410]

55. Musselman CA, Mansfield RE, Garske AL, et al. Binding of the CHD4 PHD2 finger to histone H3 is modulated by covalent modifications. Biochem J 2009;423:179–187. [PubMed: 19624289]

56. Cosgrove MS, Patel A. Mixed lineage leukemia: a structure-function perspective of the MLL1 protein. FEBS J 2010;277:1832–1842. [PubMed: 20236310]

57. Ansari KI, Mandal SS. Mixed lineage leukemia: roles in gene expression, hormone signaling and mRNA processing. FEBS J 2010;277:1790–1804. [PubMed: 20236313]

58. Terranova R, Agherbi H, Boned A, Meresse S, Djabali M. Histone and DNA methylation defects at *Hox* genes in mice expressing a SET domain-truncated form of MLL. Proc Natl Acad Sci USA 2006;103:6629–6634. [PubMed: 16618927]

59. Milne TA, Briggs SD, Brock HW, et al. MLL targets SET domain methyltransferase activity to *Hox* gene promoters. Mol Cell 2002;10:1107–1117. [PubMed: 12453418]

60. Birke M, Schreiner S, Garcia-Cuellar MP, Mahr K, Titgemeyer F, Slany RK. The MT domain of the proto-oncoprotein MLL binds to CpG-containing DNA and discriminates against methylation. Nucleic Acids Res 2002;30:958–965. [PubMed: 11842107]

61. Ayton PM, Chen EH, Cleary ML. Binding to nonmethylated CpG DNA is essential for target recognition, transactivation, and myeloid transformation by an MLL oncoprotein. Mol Cell Biol 2004;24:10470–10478. [PubMed: 15542854]

62. Allen MD, Grummitt CG, Hilcenko C, et al. Solution structure of the nonmethyl-CpG-binding CXXC domain of the leukaemia-associated MLL histone methyltransferase. EMBO J 2006;25:4503–4512. [PubMed: 16990798]

63. Cierpicki T, Risner LE, Grembecka J, et al. Structure of the MLL CXXC domain–DNA complex and its functional role in MLL-AF9 leukemia. Nat Struct Mol Biol 2010;17:62–68. [PubMed: 20010842]

64. Lee JH, Skalnik DG. CpG-binding protein (CXXC finger protein 1) is a component of the mammalian Set1 histone H3–Lys4 methyltransferase complex, the analogue of the yeast Set1/COMPASS complex. J Biol Chem 2005;280:41725–41731. [PubMed: 16253997]

65. Lee JH, Tate CM, You JS, Skalnik DG. Identification and characterization of the human Set1B histone H3–Lys4 methyltransferase complex. J Biol Chem 2007;282:13419–13428. [PubMed: 17355966]

66. Thomson JP, Skene PJ, Selfridge J, et al. CpG islands influence chromatin structure via the CpG-binding protein Cfp1. Nature 2010;464:1082–1086. [PubMed: 20393567] ▪▪Suggests that

unmethylated CpGs recruit Cfp1 and the associated methyltransferase Set1 creates new marks of H3K4me3 on the local chromatin.

67. Tsukada Y, Fang J, Erdjument-Bromage H, et al. Histone demethylation by a family of JmjC domain-containing proteins. Nature 2006;439:811–816. [PubMed: 16362057]
68. Blackledge NP, Zhou JC, Tolstorukov MY, Farcas AM, Park PJ, Klose RJ. CpG islands recruit a histone H3 lysine 36 demethylase. Mol Cell 2010;38:179–190. [PubMed: 20417597] ▪▪Demonstrates that Jumonji-domain containing histone demethylase, a H3K36me2/1 demethylase, is recruited to the nonmethylated CpG island DNA on a genome-wide scale and that this is dependent on the intrinsic Cys–X–X–Cys domain.
69. Jurkowska RZ, Anspach N, Urbanke C, et al. Formation of nucleoprotein filaments by mammalian DNA methyltransferase Dnmt3a in complex with regulator Dnmt3L. Nucleic Acids Res 2008;36:6656–6663. [PubMed: 18945701]
70. Pradhan M, Esteve PO, Chin HG, Samaranayke M, Kim GD, Pradhan S. CXXC domain of human DNMT1 is essential for enzymatic activity. Biochemistry 2008;47:10000–10009. [PubMed: 18754681]
71. Jorgensen HF, Ben-Porath I, Bird AP. Mbd1 is recruited to both methylated and nonmethylated CpGs via distinct DNA binding domains. Mol Cell Biol 2004;24:3387–3395. [PubMed: 15060159]
72. Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009;324:930–935. [PubMed: 19372391] ▪▪Among the first studies to demonstrate that 5-methylcytosine can be converted to hydroxymethylcytosine and explain the relevance of this modified base in the human genome.
73. Ono R, Taki T, Taketani T, Taniwaki M, Kobayashi H, Hayashi Y. LCX, leukemia-associated protein with a CXXC domain, is fused to MLL in acute myeloid leukemia with trilineage dysplasia having t(10;11) (q22;q23). Cancer Res 2002;62:4075–4080. [PubMed: 12124344]
74. Lorsbach RB, Moore J, Mathew S, Raimondi SC, Mukatira ST, Downing JR. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). Leukemia 2003;17:637–641. [PubMed: 12646957]
75. Dhasarathy A, Wade PA. The MBD protein family-reading an epigenetic mark? Mutat Res 2008;647:39–43. [PubMed: 18692077]
76. Hendrich B, Hardeland U, Ng HH, Jiricny J, Bird A. The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 1999;401:301–304. [PubMed: 10499592]
77. Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, Cairns BR. DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. Cell 2008;135:1201–1212. [PubMed: 19109892] ▪Suggests that in zebrafish, methyl-CpG-binding domain 4 (containing both a N-terminal methyl-CpG-binding domain and a C-terminal thymine glycosylase domain) is involved in demethylating DNA.
78. Popp C, Dean W, Feng S, et al. Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. Nature 2010;463:1101–1105. [PubMed: 20098412] ▪▪Together with [79], demonstrates that the cytidine deaminase AID has a role in DNA demethylation.
79. Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. Nature 2010;463:1042–1047. [PubMed: 20027182] ▪▪Together with [78], demonstrates that the cytidine deaminase AID has a role in DNA demethylation.
80. Kim MS, Kondo T, Takada I, et al. DNA demethylation in hormone-induced transcriptional derepression. Nature 2009;461:1007–1012. [PubMed: 19829383]
81. Sarraf SA, Stancheva I. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol Cell 2004;15:595–605. [PubMed: 15327775]
82. Lyst MJ, Nan X, Stancheva I. Regulation of MBD1-mediated transcriptional repression by SUMO and PIAS proteins. EMBO J 2006;25:5317–5328. [PubMed: 17066076]

83. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ 3rd. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev 2002;16:919–932. [PubMed: 11959841]
84. Rowe HM, Jakobsson J, Mesnard D, et al. KAP1 controls endogenous retroviruses in embryonic stem cells. Nature 2010;463:237–240. [PubMed: 20075919]
85. Fujita N, Watanabe S, Ichimura T, et al. Methyl-CpG binding domain 1 (MBD1) interacts with the Suv39h1-HP1 heterochromatic complex for DNA methylation-based transcriptional repression. J Biol Chem 2003;278:24132–24138. [PubMed: 12711603]
86. Ohki I, Shimotake N, Fujita N, et al. Solution structure of the methyl-CpG binding domain of human MBD1 in complex with methylated DNA. Cell 2001;105:487–497. [PubMed: 11371345]
87. Ho KL, McNae IW, Schmiedeberg L, Klose RJ, Bird AP, Walkinshaw MD. MeCP2 binding to DNA depends upon hydration at methyl-CpG. Mol Cell 2008;29:525–531. [PubMed: 18313390]
88. McGarvey KM, Fahrner JA, Greene E, Martens J, Jenuwein T, Baylin SB. Silenced tumor suppressor genes reactivated by DNA demethylation do not return to a fully euchromatic chromatin state. Cancer Res 2006;66:3541–3549. [PubMed: 16585178]
89. Egger G, Aparicio AM, Escobar SG, Jones PA. Inhibition of histone deacetylation does not block resilencing of p16 after 5-aza-2′-deoxycytidine treatment. Cancer Res 2007;67:346–353. [PubMed: 17210717]
90. Collins R, Cheng X. A case study in cross-talk: the histone lysine methyltransferases G9a and GLP. Nucleic Acids Res 2010;38:3503–3511. [PubMed: 20159995]
91. Clouaire T, de Las Heras JI, Merusi C, Stancheva I. Recruitment of MBD1 to target genes requires sequence-specific interaction of the MBD domain with methylated DNA. Nucleic Acids Res 2010;38(14):4620–4634. [PubMed: 20378711]
92. Walker JR, Avvakumov GV, Xue S, et al. Crystal structure of the tandem Tudor domains of the E3 ubiquitin-protein ligase UHRF1 in complex with trimethylated histone H3-K9 peptide. 2008 Epub ahead of print. 10.2210/pdb3db3/pdb
93. Rottach A, Frauer C, Pichler G, Bonapace IM, Spada F, Leonhardt H. The multi-domain protein Np95 connects DNA methylation and histone modification. Nucleic Acids Res 2009;38:1796–1804. [PubMed: 20026581]
94. Hashimoto H, Horton JR, Zhang X, Bostick M, Jacobsen SE, Cheng X. The SRA domain of UHRF1 flips 5-methylcytosine out of the DNA helix. Nature 2008;455:826–829. [PubMed: 18772888]
95. Arita K, Ariyoshi M, Tochio H, Nakamura Y, Shirakawa M. Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. Nature 2008;455:818–821. [PubMed: 18772891]
96. Avvakumov GV, Walker JR, Xue S, et al. Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. Nature 2008;455:822–825. [PubMed: 18772889]
97. Tubbs JL, Latypov V, Kanugula S, et al. Flipping of alkylated DNA damage bridges base and nucleotide excision repair. Nature 2009;459:808–813. [PubMed: 19516334]
98. Kim JK, Esteve PO, Jacobsen SE, Pradhan S. UHRF1 binds G9a and participates in p21 transcriptional regulation in mammalian cells. Nucleic Acids Res 2009;37:493–505. [PubMed: 19056828]
99. Achour M, Fuhrmann G, Alhosin M, et al. UHRF1 recruits the histone acetyltransferase Tip60 and controls its expression and activity. Biochem Biophys Res Commun 2009;390:523–528. [PubMed: 19800870]
100. Xu M, Long C, Chen X, Huang C, Chen S, Zhu B. Partitioning of histone H3-H4 tetramers during DNA replication-dependent chromatin assembly. Science 2010;328:94–98. [PubMed: 20360108]
▪▪Demonstrates that histone H3.1-H4 tetramers remain intact and are partitioned evenly during DNA replication-dependent chromatin assembly.
101. Collins RE, Northrop JP, Horton JR, et al. The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. Nat Struct Mol Biol 2008;15:245–250. [PubMed: 18264113]
102. Grewal SI, Jia S. Heterochromatin revisited. Nat Rev Genet 2007;8:35–46. [PubMed: 17173056]

103. Zhang K, Mosch K, Fischle W, Grewal SI. Roles of the Clr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin. Nat Struct Mol Biol 2008;15:381–388. [PubMed: 18345014]

104. Peisajovich SG, Garbarino JE, Wei P, Lim WA. Rapid diversification of cell signaling phenotypes by modular domain recombination. Science 2010;328:368–372. [PubMed: 20395511]

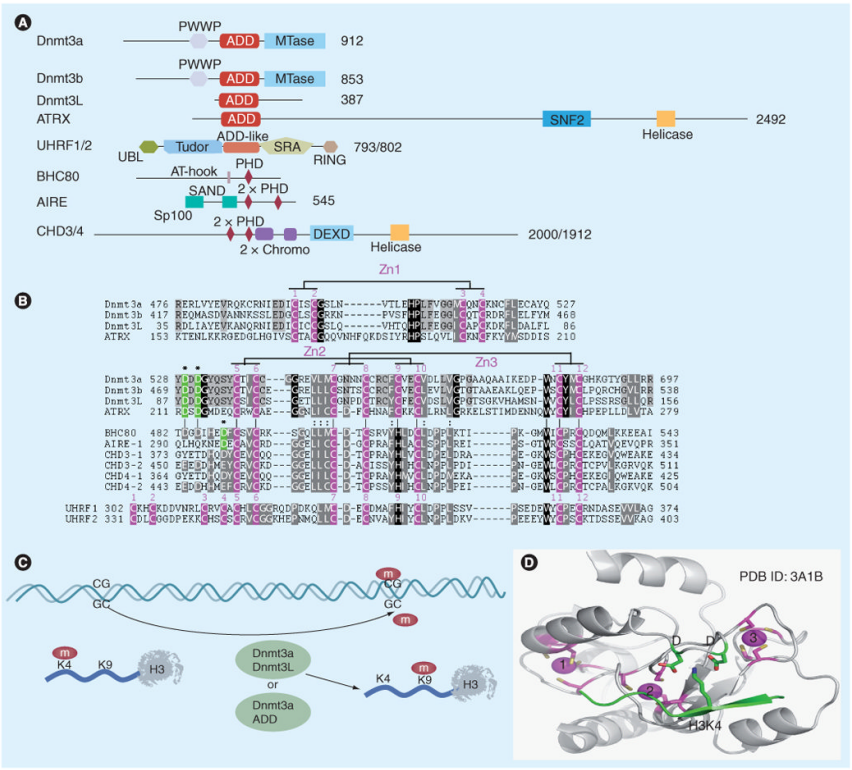

Figure 1. H3K4me0-interacting proteins

(A) Domain architecture of known H3K4me0-interacting proteins containing either ADD domain or PHD finger. ATRX interacts with histone H3.3. **(B)** Sequence alignment of ADD domains and PHD fingers known to interact with H3K4me0. **(C)** Model of the reactions that regulate DNA methylation by Dnmt3a/3L. Recognition of H3K4me0 by the ADD domain of DNMT3 directs the DNA methylation reaction. The 'm' in a circle indicates one or more methyl groups in DNA (5-methyl-cytosine) or histone lysines (methylated lysine). **(D)** Structure of the ADD domain of Dnmt3a (PDB ID: 3A1B) showing the interaction with the histone H3 tail.

ADD: ATRX–Dnmt3–Dnmt3L; Chromo: Chromosome; PDB: Protein database; PHD: Plant homeodomain; RING: Really interesting new gene; SRA: SET and RING associated.

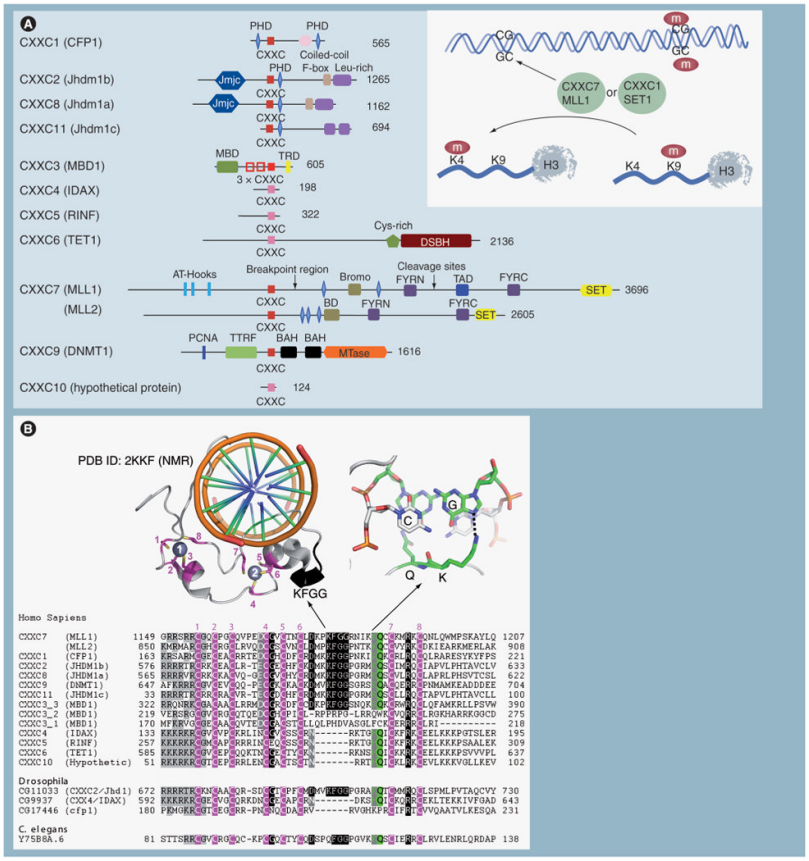

Figure 2. CpG-interacting proteins

(A) Domain architecture of CXXC domain-containing proteins. Inset: model of the H3K4 methylation reaction by MLL/SET1. CXXC domain-mediated binding to unmethylated CpGs directs the methylation of H3K4 by MLL/SET1 proteins. CXXC4 was initially named as IDAX, and decreased expression of CXXC4 promotes a malignant phenotype in renal cell carcinoma by activating Wnt signaling. CXXC5 (also known as RINF), which was initially identified in a large-scale functional proteomics mapping of human genes, activates the NF-kB and MAPK signaling pathways, and its expression correlates with retinoid-induced differentiation of leukemic cells and with cytokine-induced myelopoiesis of normal $CD34^{+}$ progenitors, is required for DNA damage-induced p53 activation and is a BMP4-regulated modulator of Wnt signaling in neural stem cells. **(B)** Ribbon diagram (top left) and stick diagram (top right) of the MLL1 CXXC domain in complex with unmethylated DNA. Note the recognition of CpG by a Lys–Gln dipeptide in the major groove. Sequence alignment of CXXC domains including candidates from *Drosophila* and *Caenorhabditis elegans* (Bottom).

BAH: Bromo-adjacent homology domain; CHD: Chromodomain-helicase-DNA-binding protein; CXXC: Cys–X–X–Cys; DSBH: Double-stranded β-helix; FYRC: FY-rich domain C-terminal region; FYRN: FY-rich domain N-terminal region; IDAX: Inhibiton of the Dvl and Axin complex in the Wnt signaling pathway; MBD: Methyl-CpG-binding domain; PCNA: Proliferating cell nuclear antigen; PHD: Plant homeodomain; RINF: Retinoid-inducible nuclear factor; SET: Su(var)3-9, Enhancer-of-zeste, Trithorax; TTRF: Target to replication foci.

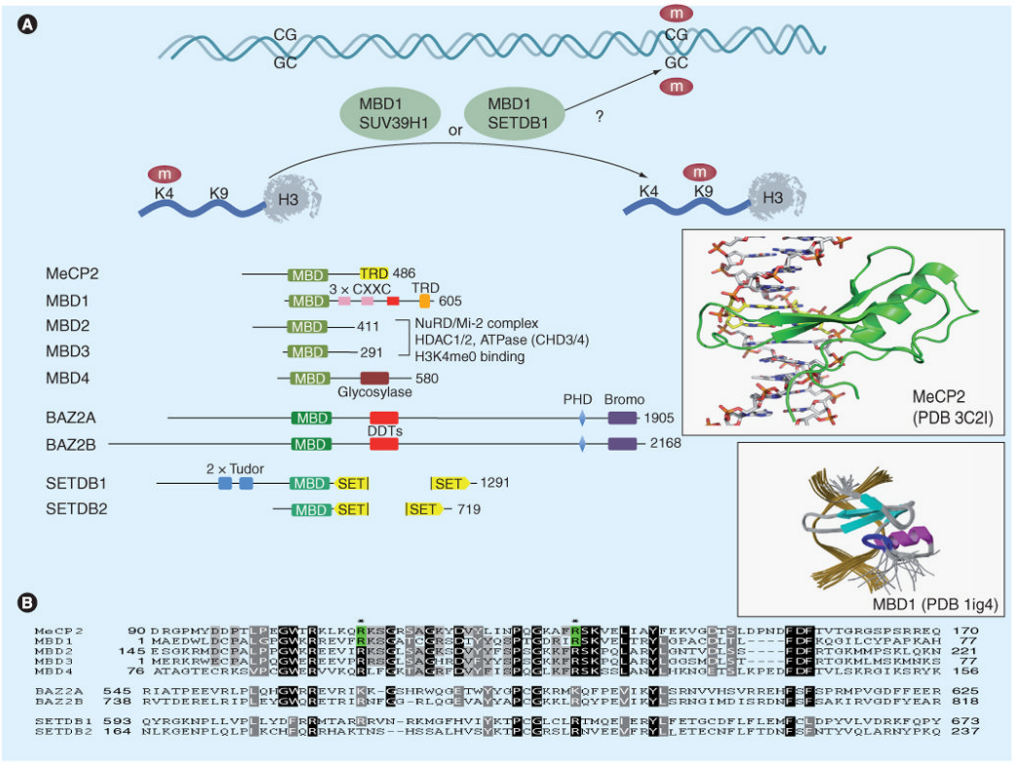

Figure 3. Methyl-CpG-binding domain-containing proteins

(A) Model of the reactions that regulate H3K9 methylation by SUV39H1 or SETDB1. Recognition of methylated CpGs by the MBD of MBD1 directs methylation of H3K9me3. Inset: MBD structures of MeCP2 and MBD1 in complex with methyl-CpG DNA, respectively. **(B)** Sequence alignment of known and putative MBD domains. BAZ2-A and -B are two related proteins containing a MBD, DDT, PHD-type zinc finger and bromodomain. DDT is predicted to be a DNA binding domain (~60 residues in length). BAZ2A, also known as TTF-I interacting peptide 5 or TIP5; hWALp3, is a component of the chromatin remodeling complex NoRC, a complex that mediates silencing of a fraction of rDNA.

DDT: DNA-binding homeobox and different transcription factors; HDAC: Histone deacetylase; MBD: Methyl-CpG-binding domain; PDB: Protein database; PHD: Plant homeodomain; SET: Su(var)3-9, Enhancer-of-zeste, Trithorax.

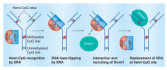

Figure 4. Putative model of the coupling of UHRF1 and Dnmt1 to maintain the fidelity of DNA methylation during DNA replication

The binding of the SRA domain of UHRF1 to hemi-methylated CpG sites generated by new strand synthesis recruits Dnmt1, allowing for displacement and subsequent methylation of the daughter strand.

SRA: SET and RING associated.