8556–8575 *Nucleic Acids Research, 2016, Vol. 44, No. 18* *Published online 12 August 2016*
doi: 10.1093/nar/gkw723

SURVEY AND SUMMARY

Allosteric control of mammalian DNA methyltransferases – a new regulatory paradigm

Albert Jeltsch[1,*] and Renata Z. Jurkowska[2,*]

[1]Institute of Biochemistry, Pfaffenwaldring 55, Faculty of Chemistry, University of Stuttgart, D-70569 Stuttgart, Germany and [2]BioMed X Innovation Center, Im Neuenheimer Feld 583, D-69120 Heidelberg, Germany

Received July 06, 2016; Revised August 03, 2016; Accepted August 08, 2016

ABSTRACT

In mammals, DNA methylation is introduced by the DNMT1, DNMT3A and DNMT3B methyltransferases, which are all large multi-domain proteins containing a catalytic C-terminal domain and an N-terminal part with regulatory functions. Recently, two novel regulatory principles of DNMTs were uncovered. It was shown that their catalytic activity is under allosteric control of N-terminal domains with autoinhibitory function, the RFT and CXXC domains in DNMT1 and the ADD domain in DNMT3. Moreover, targeting and activity of DNMTs were found to be regulated in a concerted manner by interactors and posttranslational modifications (PTMs). In this review, we describe the structures and domain composition of the DNMT1 and DNMT3 enzymes, their DNA binding, catalytic mechanism, multimerization and the processes controlling their stability in cells with a focus on their regulation and chromatin targeting by PTMs, interactors and chromatin modifications. We propose that the allosteric regulation of DNMTs by autoinhibitory domains acts as a general switch for the modulation of the function of DNMTs, providing numerous possibilities for interacting proteins, nucleic acids or PTMs to regulate DNMT activity and targeting. The combined regulation of DNMT targeting and catalytic activity contributes to the precise spatiotemporal control of DNMT function and genome methylation in cells.

INTRODUCTION

In mammals, DNA methylation occurs at the C5 position of cytosine residues, primarily in CpG dinucleotides, 60–80% of which are methylated in a tissue and cell type specific pattern (1–5). The correct methylation pattern is essential for development and differentiation of cellular phenotypes, and aberrant DNA methylation is a driving force in the onset and progression of several diseases, in particular cancer (6–9). Recently, the presence of 6-methyladenine in mammalian DNA has been discovered (10–13), but reported m6A levels are low, its biological roles are still unclear and the responsible enzymes have not been identified yet; therefore, it will not be considered further in this review.

DNA-(cytosine C5)-methylation is introduced during early development of mammals and maturation of germ cells by two related DNA methyltransferases (MTases), DNMT3A and DNMT3B, with the help of the stimulatory factor DNMT3L. DNMT3A and DNMT3B were classically designated as *de novo* methyltransferases, because they do not show any significant preference between hemimethylated and unmethylated DNA substrates (14,15). They are highly expressed in undifferentiated cells and germ cell precursors, but present at much lower levels in somatic cells. In the cell nucleus, DNMT3 enzymes localize to pericentromeric heterochromatin (16,17), where they are tightly bound to nucleosomes containing methylated DNA (18,19). After establishment, methylation patterns are preserved for the rest of the life of an organism, with small tissue-specific changes. During DNA replication, fully methylated CpG sites are converted into hemimethylated sites, which are then re-methylated mainly by the so-called maintenance methyltransferase DNMT1. This enzyme is present at the replication forks and preferentially methylates hemimethylated CpG sites, thereby copying the methylation pattern from the parental DNA strand onto the newly synthesized daughter strand (3–4,20–22). DNMT1 is ubiquitously and highly expressed in proliferating cells, representing the major DNA methyltransferase activity in somatic tissues throughout mammalian development and is present only at low levels in non-dividing cells (23). The sub-nuclear localization of

*To whom correspondence should be addressed. Tel: +49 711 685 64390; Fax: +49 711 685 64392; Email: albert.jeltsch@ibc.uni-stuttgart.de
Correspondence may also be addressed to Renata Z. Jurkowska. Tel: +49 6221 426 11 42; Fax: +49 6221 426 11 22; Email: jurkowska@bio.mx

DNMT1 changes dynamically during the cell cycle, it is diffusely distributed in the nucleus during interphase, and localizes to the replication foci in S-phase creating a characteristic punctuate pattern (24,25).

However, it has been recently recognized that this classification of DNA MTases into *de novo* and maintenance methyltransferases is an oversimplification and that DNA methylation is better described as a dynamic process of ongoing methylation and demethylation, in which DNMT1 and DNMT3 enzymes together play roles in both *de novo* and maintenance methylation (1,26–27). This conceptual change in the perception of DNA methylation as a dynamic process has important implications entailing that it is the regulation and targeting of DNMTs that controls the methylation state of each CpG site, and not the conceptually simple copy process brought about by DNMT1 after DNA replication. However, the preference of DNMT1 for the methylation of hemimethylated CpG sites is certainly an important factor in the control of cellular DNA methylation patterns. Consequently, the complex role of DNA methylation in human biology critically depends on the dynamic regulation and targeting of DNMTs by posttranslational modifications (PTMs), as well as by their interaction with other chromatin factors (e.g. histones), other interacting proteins or nucleic acids.

The general architecture of all three mammalian DNMTs is similar. They all are multi-domain proteins, in which two functional parts can be distinguished, a large N-terminal regulatory part and a smaller C-terminal part, harboring the catalytic center (Figure 1) (3,20–21). The C-terminal domains of DNMTs contain 10 conserved amino acid motifs characteristic for DNA-(cytosine-C5)-MTases. They fold into a widely conserved structure found in all enzymes of this class, the so called 'AdoMet-dependent MTase fold', which consists of a mixed 7-stranded β-sheet formed by 6 parallel β strands and a 7th strand inserted into the sheet in an anti-parallel orientation between strands 5 and 6. Six alpha helices surround the central β-sheet on both sides (20,22). The C-terminal domain of DNMTs is involved in AdoMet binding, DNA recognition and binding, target base flipping and catalysis. It contains a non-conserved region, the so-called target recognition domain (TRD) that is involved in DNA recognition and specificity.

The catalytic mechanism of DNA-(cytosine C5)-methyltransferases has been elucidated using prokaryotic DNA MTase as model systems more than two decades ago (20,28). The transfer of the methyl group from the AdoMet to the C5 position of cytosine residues is enabled by the rotation of the target base out of the DNA double helix and its incorporation into a hydrophobic pocket in the active center of the enzyme next to the AdoMet, in a mechanism called base flipping. The base flipping is common to all DNA methyltransferases and it has recently been observed in the crystal structure of DNMT1, the first mammalian DNMT co-crystallized with its substrate DNA (29). The methylation of the C5 position of cytosine follows a two-step reaction mechanism. First, a catalytic cysteine residue located in the PCQ motif attacks the C6 position of the cytosine ring, leading to the formation of a covalent bond between the enzyme and the substrate base. This process leads to the activation of the C5 position and methyl group transfer. The addition of the methyl group to the base is followed by a deprotonation of the C5 atom, which resolves the covalent bond between the enzyme and the base in an elimination reaction (20,28). Kinetic isotope effects have recently confirmed this two-step mechanism for DNMT1 (30). In addition, mutations of the key catalytic residues in DNMT3A were found to reduce its catalytic activity, although in some cases unexpected residual activity was observed (31–33).

The N-terminal parts of DNMTs, which are different between the DNMT1 and the DNMT3 family of enzymes, comprise several domains with regulatory functions. They guide the nuclear localization of the enzymes, mediate their interaction with other proteins, regulatory nucleic acids (like non-coding RNAs) and chromatin, and are subject to post-translational modifications. Moreover, recent structural and biochemical data have demonstrated that the arrangement of these domains plays a central role in the regulation of the biological functions of DNMTs, because they are involved in the allosteric regulation of the enzymes' activity, specificity and localization.

STRUCTURE, MECHANISM AND REGULATION OF DNMT1

Domain composition of DNMT1

DNMT1 is a large enzyme, occurring in different isoforms generated by alternative splicing and the usage of an alternative promoter (3,21). DNMT1 contains multiple functional domains located in its N-terminal part that is joined to the C-terminal part by a flexible linker composed of lysine-glycine (KG) repeats (Figure 1). The N-terminal part serves as a platform for the assembly of various proteins involved in the control of DNA replication and repair, chromatin structure and gene regulation. It is composed of several domains that are listed here in the order from the N- to the C-terminus. The DMAP1 (DNA methyltransferase-associated protein 1) interaction domain contacts the transcriptional repressor DMAP1. With this interaction, DNMT1 targets DMAP1 to replication foci throughout S phase (34). DMAP1 influences maintenance methylation by DNMT1 in early development (35) and affects the stability of DNMT1 in cells (36). The PCNA (proliferating cell nuclear antigen) binding domain (PBD) is involved in the targeting and tethering of DNMT1 to the replication fork during S phase that supports DNA methylation in cells (37,38). It also contains an AT-hook-like DNA binding motif (39). The replication foci targeting domain (RFTD) is involved in the targeting of DNMT1 to replication foci (25) and centromeric chromatin (24). It interacts with UHRF1 (ubiquitin-like with PHD and ring finger domains 1) protein (40,41) that binds to hemimethylated DNA and chromatin modifications and also contains a RING E3 ligase domain. The RFTD domain is followed by the CXXC domain, which binds unmethylated DNA (42–44) and comprises a zinc finger containing eight cysteine residues and two zinc ions. Finally, the BAH1 and BAH2 (bromo-adjacent homology 1 and 2) domains are necessary for the folding of DNMT1. It is currently unknown whether they have further functional roles, although

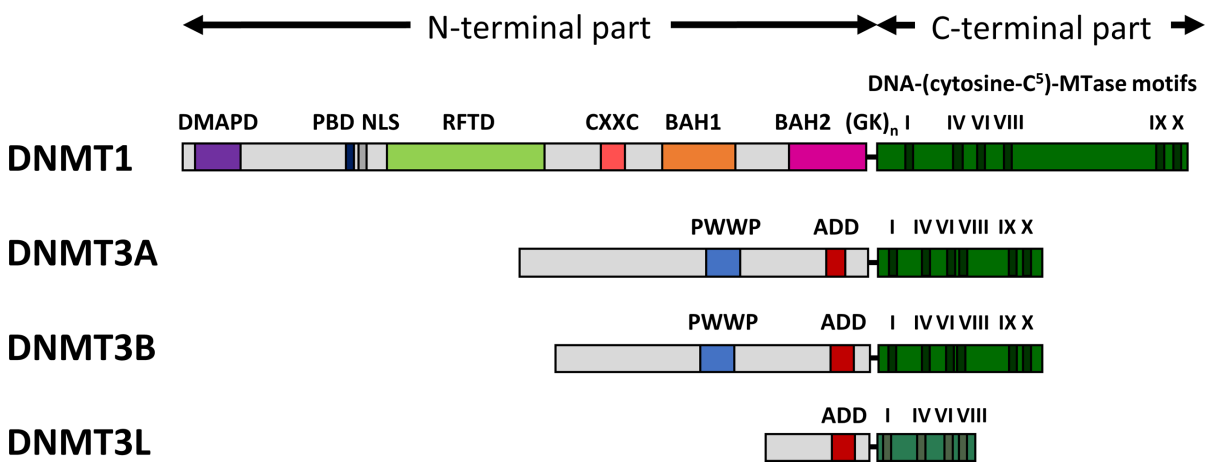

Figure 1. Domain structure of the mammalian DNMT enzymes. The human DNMT1, DNMT3A, DNMT3B and DNMT3L proteins consist of 1616, 912, 853 and 387 amino acid residues, respectively. Abbreviations used: DMAPD – DNA methyltransferase associated protein 1 interacting domain, PBD – PCNA binding domain, NLS – Nuclear localization signal, RFTD – Replication foci targeting domain, CXXC – CXXC domain, BAH1 and BAH2 – bromo-adjacent homology domains 1 and 2, GK_n – glycine lysine repeats, PWWP – PWWP domain, ADD – ATRX-DNMT3-DNMT3L domain.

chromatin targeting by a BAH domain has been described in plant DNMTs (45).

Structure of a catalytically active DNMT1 fragment with bound substrate DNA

The structural arrangement of the particular domains in DNMT1 has recently been revealed by several crystallographic studies (29,43,46–48) (Figure 2). In summary, these studies demonstrated that the various domains in the N-terminal part of DNMT1 surround and contact the C-terminal catalytic domain, indicating that the proper folding of the catalytic domain likely requires its N-terminal domains. Unexpectedly, the crystal structures also revealed that the enzyme undergoes large domain rearrangements, which allosterically regulate its catalytic activity (Figure 2).

A C-terminal fragment of DNMT1 comprising the BAH domains and the catalytic domain showed an open conformation of the catalytic center, in which the enzyme was able to bind and methylate substrate DNA (29) (Figure 2A). The elucidation of this complex structure represented a breakthrough in the field, as it provided the first example of a mammalian DNMT structure solved with its substrate DNA bound in the active site. It confirmed that the C-terminal domain of DNMT1 adopts the canonical AdoMet-dependent MTase fold. As expected, it showed the target base flipped out of the DNA helix and bound to the enzyme in a manner reminiscent of other DNA MTases. Moreover, several contacts of the enzyme to the target CpG site were identified, which were confirmed in kinetic studies as essential for enzymatic activity and recognition of the CpG site (49). The BAH1 and BAH2 domains of the mouse DNMT1 (mDNMT1) are connected by an α-helix and are arranged in a dumbbell like manner on the left and right side of the catalytic domain. Despite low sequence conservation, both BAH domains adopt a common distorted barrel structure comprising a mixture of 10 parallel and antiparallel β-strands and one helix. The close contacts between the BAH domains and the catalytic domain suggest that the isolated catalytic domain would probably be misfolded, which explains why it is catalytically inactive in an isolated form (50,51).

As mentioned above, DNMT1 shows a preference for hemimethylated DNA as compared to unmethylated substrates, supporting its role as maintenance MTase (29,44,49–50,52). Its intrinsic preference for hemimethylated DNA has been estimated to about 30- to 40-fold (53), but it largely depends on the exact substrate sequence, its length and the reaction conditions. Mechanistically, the specificity of DNMT1 is mediated by the binding of the methyl group of the hemimethylated CpG site into a hydrophobic pocket in the C-terminal domain of the methyltransferase formed by C1501, L1502, W1512, L1515 and M1535, which favors the binding of hemimethylated CpG sites (29). Further details of this process and eventual conformational changes after binding to an unmethylated CpG site could only be uncovered, if a structure of DNMT1 with an unmethylated DNA bound to the active center became available. The recognition of the 5mC-G base pair is further mediated by M1535, K1537, Q1538 and R1237, which form side-chain and backbone H-bonds to the edges of the base pair in the major and minor groove (29). These interactions explain why the 5mC and the corresponding G in the target DNA strand are very accurately recognized by DNMT1 and cannot be exchanged by other nucleotides (49).

Moreover, the DNMT1 structure with DNA bound in the active center also uncovered additional unforeseen rearrangements in the DNMT1–DNA structure, including the formation of a non-Watson–Crick base pair of the orphan G residue with a G flanking the CpG site. The (then orphaned) C of the flanking G:C base pair was rotated out of the DNA helix in a direction roughly opposite to the target C flipping. However, kinetic experiments could not provide evidence for this reshuffling of base pairs and the double base flipping (49), suggesting that this structure may still not capture the true transition state of the methylation reaction.

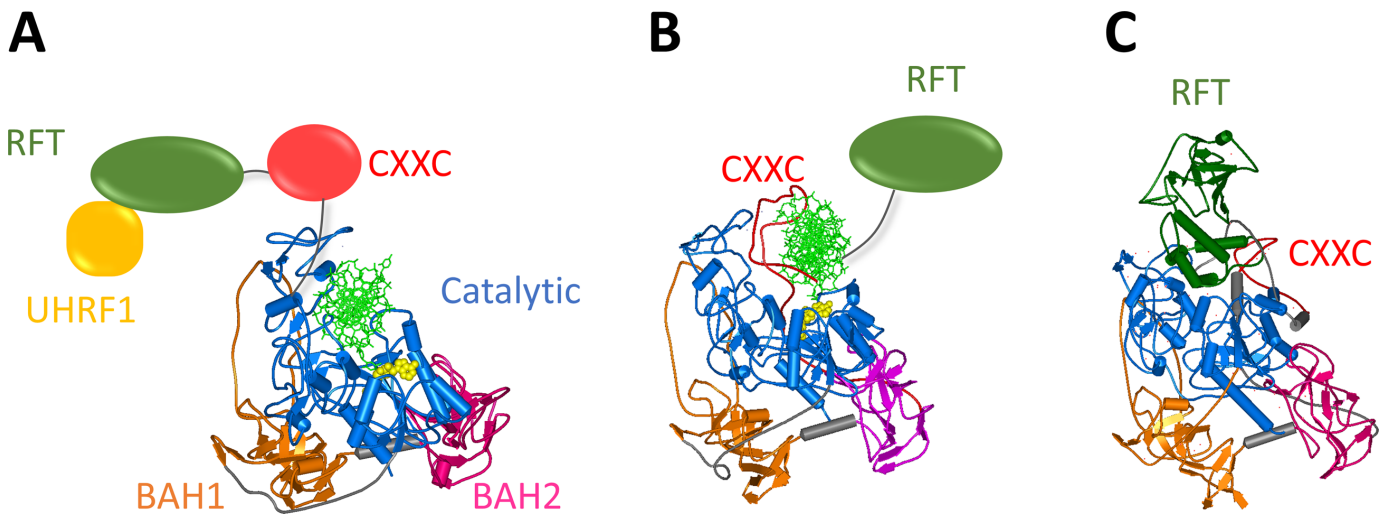

Figure 2. Structures of DNMT1 with different N-terminal domains. The RFT, CXXC, BAH1, BAH2 and catalytic domains are shown in dark green, red, orange, purple and blue, respectively. (**A**) DNMT1 in an active conformation with DNA (light green) bound in the active site (29). Removal of the autoinhibitory RFTD can be triggered by UHRF1 (40,41). (**B**) DNMT1 with unmethylated DNA bound to the autoinhibitory CXXC domain (43). (**C**) DNMT1 with the RFT domain blocking access to the active site (46).

DNMT1 structures reveal allosteric control of its activity

The structure of a larger DNMT1 C-terminal fragment additionally containing the CXXC domain was solved in complex with an unmethylated DNA. Unexpectedly, the DNA did not bind to the catalytic domain, but a CpG site-specific DNA binding to the CXXC domain was observed (43) (Figure 2B). The CXXC domain of mDNMT1 adopts a crescent-like structure. It contains two short helical segments that bind two zinc ions; each of them is coordinated by four conserved cysteine ligands provided by two CGXCXXC motifs and two distal cysteine residues. The CXXC domain binds DNA by approaching the major groove, with the protein located perpendicularly to the DNA axis. This allows a loop segment (consisting of R684-S685-K686-Q687) to form base-specific contacts and phosphodiester interactions that contribute to the recognition of an unmethylated CpG site (43).

This observation led to the proposal that the CXXC domain of DNMT1 might have an autoinhibitory function and that it would act as a specificity filter by preventing unmethylated DNA from accessing the active site. Kinetic experiments with the truncated DNMT1 used for the structural analysis indeed revealed a clear influence of the CXXC domain on the specificity of DNMT1 (43). Surprisingly, similar experiments conducted with the full-length DNMT1 did not provide evidence for a role of the CXXC domain in the specificity of DNMT1 (44), indicating that the presence of additional domains may influence the domain arrangement. Further experiments are needed to resolve this interesting discrepancy.

Finally, another crystal structure of an almost complete DNMT1 fragment, including the RFT domain but without DNA was solved (46) and the structure of the isolated RFT domain has been obtained as well (47). The RFT domain consists of a zinc-binding motif, followed by a six-stranded β-barrel and a helical bundle, consisting of five closely associated alpha helices (47). The DNMT1 structure containing the RFT domain provided additional seminal insights into the mechanism of DNMT1, because unexpectedly, it showed that the RFT domain is deeply inserted into the DNA-binding pocket of the catalytic domain, thereby inhibiting the enzyme (46) (Figure 2C). The autoinhibitory binding of the RFT domain to the catalytic domain is stabilized by several hydrogen bonds between both protein parts and ionic interactions between the negative electrostatic potential of the RFT domain and the positive potential of the DNA binding site in the catalytic domain (46). Autoinhibition of the activity of DNMT1 by the RFT domain was confirmed in biochemical studies (46,47). Interestingly, engineering of this interface led to an alteration of the DNMT1 conformation, generating a methyltransferase that was hyperactive *in vitro* and in cells (54).

Mechanism of DNMT1

Several *in vitro* studies have shown that DNMT1 is a highly processive enzyme, able to methylate long stretches of hemimethylated DNA without dissociation from the substrate (52,55–56). During processive methylation, methyl groups are introduced only in one strand of the DNA, which indicates that DNMT1 does not exchange DNA strands while moving along its substrate (55). Due to its high processivity, DNMT1 is a very effective enzyme, ideally suited to follow DNA replication and methylate the newly synthetized DNA strand before the chromatin is reassembled. The structure of DNMT1 with bound substrate DNA (29) showed that the enzyme enwraps the DNA, which enables it to slide along the substrate and catalyze several successive methylation reactions without dissociation from the DNA. Therefore, the ability of DNMT1 to adopt open and closed conformations might not only be involved in the regulation of its activity, but also of its processivity, because the ability of the enzyme to adopt a closed conformation after DNA binding may help preventing its dissociation from the DNA and thereby increase the processivity.

Allosteric regulation and targeting of DNMT1 by interacting partners

As described above, accumulating experimental evidence indicates that the autoinhibitory mechanism of the RFT domain plays a central role as an allosteric trigger in DNMT1 (Figures 2 and 3). The flexible arrangement of different domains in DNMT1 is controlled by linker regions, which form interactions with surface clefts of the domains. Both the linkers and the surface clefts are subject to many PTMs in DNMT1, including phosphorylation, acetylation and ubiquitination, which might directly control the positioning of these domains in DNMT1 (Figure 4). Moreover, binding of individual domains to various interacting partners has been shown to alter the domain arrangement and the activity of DNMT1.

The DNMT1–PCNA interaction. The sub-nuclear localization of DNMT1 changes dynamically during the cell cycle (3,21). It is diffusely distributed in the nucleus during interphase when cells are not replicating, but in the early and mid S-phase DNMT1 localizes to the replication foci in cells actively synthesizing DNA, creating a characteristic punctuate pattern. The targeting of DNMT1 to replication foci during S-phase is mediated by the PCNA binding domain (37), the replication foci targeting domain (25) and the BAH domains (57) (Figure 3). Through the PBD domain, DNMT1 directly interacts with PCNA, the processivity factor of the DNA replication machinery (24). DNMT1 and PCNA co-localize *in vivo*, indicating that PCNA might recruit DNMT1 to the replication fork and load it onto DNA. Indeed, expression of a truncated DNMT1, which lacks parts of the PBD domain, led to a delay in the re-methylation of DNA after replication (38). However, it did not cause massive defects in DNA methylation, indicating that the interaction of PCNA with DNMT1 contributes to the efficiency of DNA re-methylation, but is not indispensable for this process. In addition, *in vitro* experiments provided evidence that the interaction with PCNA increases DNA binding and catalytic activity of DNMT1, suggesting that it may also exert allosteric effects on the catalytic domain (58).

During progression of the S-phase, the sub-nuclear pattern of DNMT1 localization changes from small, punctuate and abundant structures in early S-phase to fewer, large, toroidal structures in late S phase, which co-localize with the late-replicating heterochromatic satellite DNA (24–25,59), and some DNMT1 remains associated with centromeric heterochromatin in the late S and G2 phases, even after heterochromatin replication. The interaction with heterochromatin is mediated by the PBD domain of DNMT1 in a replication-independent manner (24) and it is also at least in part mediated by the interaction of DNMT1 with UHRF1, as described in the next section.

The DNMT1–UHRF1 interaction. Another pathway of DNMT1 targeting was discovered about 10 years ago with the finding that UHFR1 is required for the maintenance of DNA methylation in mammals (60,61). UHRF1 specifically binds to hemimethylated DNA via its SET and RING associated (SRA) domain (60,62–64) and its localization to replicating heterochromatin is dependent on the presence of hemimethylated DNA (61). UHFR1 co-localizes with DNMT1 and PCNA at replicating heterochromatic regions during mid to late S-phase and the association of DNMT1 with chromatin is lost in UHFR1 knockout (KO) cells (60,61). Remarkably, the phenotype of the UHFR1 KO in mice mimics that of the DNMT1 KO, as UHRF1-deficient embryos die shortly after gastrulation and show significantly reduced levels of DNA methylation genome-wide (61), indicating that UHRF1 has an essential role in the maintenance of DNA methylation. These observations led to a model that UHFR1 recruits DNMT1 to replicated hemimethylated DNA to facilitate its efficient re-methylation (65) (Figure 3).

Later, it was found that UHRF1 is part of a complex chromatin interaction network (Figure 3) and two additional domains of UHRF1, the tandem Tudor domain (TTD) and the plant homeodomain (PHD) further contribute to the maintenance of DNA methylation. TTD binds histone 3 tails methylated at lysine 9 (H3K9me3) and unmethylated at lysine 4 (66,67), whereas the PHD domain binds to H3 tails unmodified at arginine 2 (H3R2) (68–70). The interaction with H3K9me3 is required for the localization of UHRF1 to heterochromatin and for maintenance DNA methylation, since a mutation in TTD, which prevents binding of UHRF1 to H3K9me3, abolished both functions (66,67). Similarly, disruption of H3R2 binding abolished DNA methylation by DNMT1 in cells (71). These data indicate that the coordinated recognition of two histone marks, H3K9me3 and H3R2, by UHRF1 as well as its interaction with hemimethylated DNA are all necessary for guidance of DNMT1 to DNA and subsequent maintenance of DNA methylation patterns (72,73). Recently, the central role of the H3K9me2/UHRF1/DNMT1 axis in the control of DNA methylation has also been demonstrated in the differentiation of ES cells (74).

In line with the general model of combined targeting and allosteric activation of DNMTs by interactors proposed here, UHRF1 was also shown to directly stimulate the catalytic activity of DNMT1, by interacting with the RFT domain of the MTase and relieving the auto-inhibition mediated by this domain (40,41). Moreover, the RING domain of UHRF1 has been shown to ubiquitinate H3 at K18 and K23 (71,75). Ubiquitinated H3 is bound by DNMT1, leading to the stimulation of its methyltransferase activity. Finally, UHRF1 is involved in the ubiquitination of DNMT1 that reduces DNMT1's stability (see below). All these observations demonstrate that UHRF1 is a multifaceted regulator of DNMT1 activity, targeting and stability (Figure 3). Interestingly, a structural and biochemical study has shown that UHRF1 also employs a domain conformational change triggered by binding of hemimethylated DNA to enhance H3K9me3 binding (76), indicating that conformational changes dictate the behavior of different subunits of chromatin complexes.

Binding of the DNMT1–RFTD to H3-ubiquitination. Recent studies demonstrated the role of histone H3 ubiqitination in DNMT1 function. DNMT1 associates with the ubiquitylated H3 through its RFT domain and this interaction contributes both to the targeting of DNMT1 to repli-

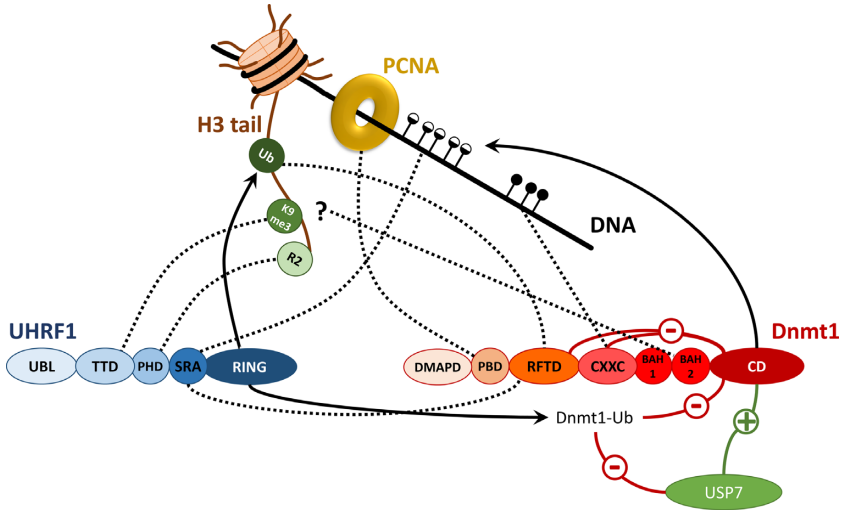

Figure 3. Regulatory mechanisms controlling the activity, targeting and stability of DNMT1. The figure illustrates the complex interplay between DNMT1, UHRF1, USP7, PCNA and chromatin. Enzymatic activities are indicated by arrows. Binding ('reading') interactions are symbolized by dotted lines. For details cf. the text.

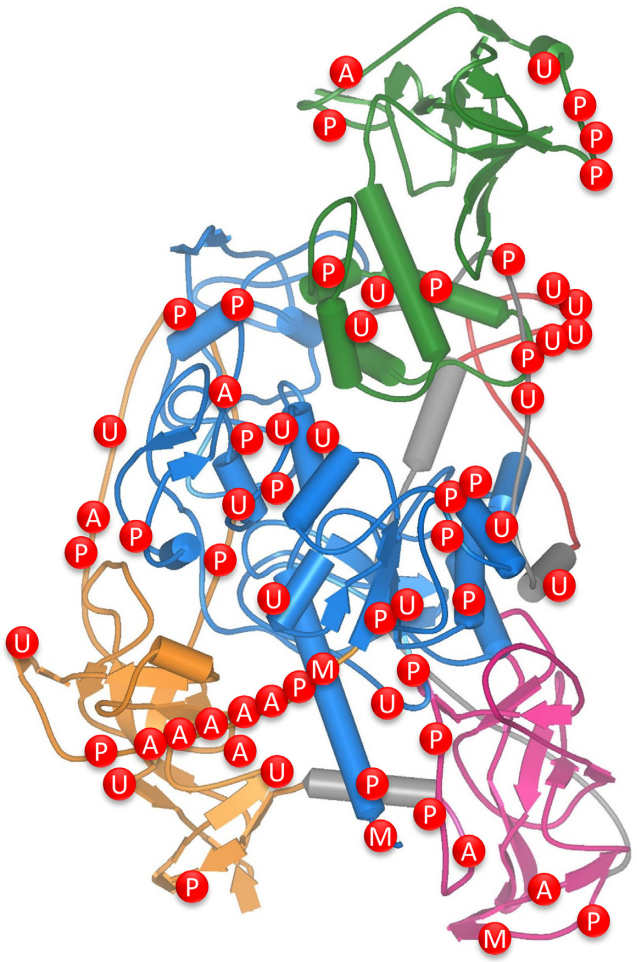

Figure 4. Representation of posttranslational modifications (PTMs) found in human DNMT1 on the structure of murine DNMT1 (46). Phosphorylations, acetylations, methylations and ubiquinations retrieved from Phosphosite Plus are represented by red circles labeled with P, A, M or U, respectively. The DNMT1 domains are colored as in Figure 2C.

cation foci and to the activation of the enzyme (71,75), providing yet another example of a concerted regulation of multiple aspects of DNMT's function. The ubiqitination of the H3 tail is introduced through the E3 ligase activity of the UHRF1 RING domain (71,75). Ubiquitination has been observed at K18 and K23 of H3, and also on DNMT1 itself, but it is unclear at present how these findings are related. These data highlight another important connection between the DNMT1 and UHRF1-mediated chromatin interactions, which is essential for an efficient maintenance methylation to occur (Figure 3). Whether conformational changes of DNMT1 are involved in this process is not known at present.

The DNMT1–USP7 interaction. Several studies showed that Ubiquitin-specific protease 7 (USP7), also known as Herpes virus-associated ubiquitin-specific protease (HAUSP), binds to DNMT1 and increases its stability through deubiquitination (77–79) (Figure 3). DNMT1 and USP7 form a stable, soluble complex that associates with UHRF1 as a trimeric complex on chromatin (78). The crystal structure of a human DNMT1 fragment starting with the CXXC domain in complex with USP7, but without DNA revealed that the interaction of both proteins is mainly mediated by the KG linker of DNMT1 and an acidic pocket near the C-terminus of USP7. Mutations of these acidic residues in USP7 and acetylation of the lysine residues of the KG linker in DNMT1 disrupted the interaction between DNMT1 and USP7, leading to increased proteasomal degradation of DNMT1 (80). Interestingly, binding of USP7 also increased the activity of DNMT1 *in vitro* (78), indicating that USP7 acts as a general positive factor of DNMT1, by increasing the stability and activity of the methyltransferase. It is currently unknown, whether USP7 binding affects the autoinhibition of DNMT1 by the N-terminal domain. While no conformational changes were observed in the structure of USP7 bound to a DNMT1 fragment starting with the CXXC domain (80), the binding of USP7 to the KG linker region suggests

that it might restrict the ability of the full-length DNMT1 to adopt certain conformations in solution. In addition, USP7 deubiquitinates other chromatin-associated proteins, including histone H2B and the histone acteyltransferase Tip60, and it prevents autoubiquitinylation of UHRF1, which increases its stability (78), indicating that USP7 plays an important general role in epigenetic regulation (81).

Regulation of activity and specificity of DNMT1 by nucleic acid binding

DNMT1 contains multiple DNA binding sites, which contribute to the allosteric regulation of its activity and specificity. Several early studies showed that the enzyme has reduced specificity in the presence of methylated DNA (50,82–85). This effect was due to an increase in the methylation rate of of unmethylated DNA, while the methylation of hemimethylated DNA was weakly inhibited (50,52). These data suggest that binding of the methylated DNA to the N-terminal part of the enzyme induces an allosteric activation and relaxation of specificity, which stimulate the methylation of unmethylated DNA. However, the molecular mechanism of this allosteric activation of DNMT1 is currently unknown; the CXXC domain (50) and the residues 284–287 of the murine DNMT1 (85) have been implicated in it, suggesting that DNA binding to the CXXC domain may mediate the effect. In addition, a general inhibitory effect of unmethylated DNA on DNMT1 was demonstrated in several studies (84,86–87). The binding site for this substrate inhibition effect was localized within the first 501 amino acids of DNMT1 (88). Additional evidence suggests that binding of methylated DNA to the N-terminal inhibition site also causes de-repression of the enzyme (88). Whether these various inhibition and stimulation effects are due to binding to the same or different sites and to what extend different DNAs compete for the different sites is not clear. Moreover, the involvement of direct binding of these DNAs to the catalytic domain can also not be excluded.

Interestingly, many studies found that binding of DNMT1 to unmethylated DNA at an allosteric site reduces its activity, while binding to methylated DNA increases it. This observation could be related to the bimodal nature of DNA methylation patterns found in the human genome that mainly comprises highly methylated or completely unmethylated regions (89–91). Propagation of such bimodal methylation patterns is supported by the allosteric binding of DNA to secondary sites in DNMT1, because DNMT1 would be activated on methylated regions and inactivated on unmethylated DNA, which would lead to a further gain of methylation at highly methylated regions and loss of methylation at lowly methylated regions.

Besides DNA, DNMT1 is able to bind to different noncoding RNAs. Initial studies revealed that DNMT1 purified from insect cells contained inhibitory RNA (92). Later, it was discovered that RNA binding regulates the activity of DNMT1 in a locus specific manner (93). A long noncoding RNA (ncRNA) originating from the CEBPA locus was shown to bind and inhibit DNMT1, and prevent the methylation of this locus. Similar effects were observed for several other loci on the genomic level (93). Based on these findings, the authors proposed a model, in which the ncRNAs transcribed at one locus shield this locus from methylation. Thereby, the expression of the locus would be amplified, causing a positive feedback loop that keeps the locus unmethylated and transcriptionally active. Recently, it was also reported that DNMT1 binds to microRNAs (miRNAs), like miR-155-5p (94). Similarly to the long ncRNAs, miRNAs function as inhibitors of DNMT1 and the transfection of miRNAs into cells influences cellular DNA methylation (94). RNA binding was mapped to the catalytic domain of DNMT1 (93,94), and the miRNAs were shown to act as DNA competitive inhibitors (94), suggesting that the inhibition of DNMT1 by RNAs is based on a direct competition between the RNA and the substrate DNA for access to the catalytic center. However, it is also conceivable that the additional DNA binding sites described above could bind regulatory RNAs, leading to allosteric effects. Hence, important features of the interaction of DNMT1 with regulatory DNA and RNA are not yet understood at a molecular level and deserve further attention.

Regulation of DNMT1 by PTMs

DNMT1 undergoes several PTMs, including phosphorylation, methylation, ubiquitination, acetylation and sumoylation. Following the initial identification of serine 515 as a major phosphorylation site in murine DNMT1 purified from insect cells (95), several phosphorylated serine, threonine and tyrosine residues have been identified in targeted and high-throughput proteomics approaches with DNMT1 purified from human cells. Currently, more than 100 PTM sites have been mapped on human and mouse DNMT1 (http://www.phosphosite.org) (Figure 4). They localize to the crucial parts of the methyltransferase: on the surfaces of the DNMT1 domains, where they can influence the interaction with other proteins, in the linker regions connecting the domains, where they can directly affect the conformation of the enzyme, and in the catalytic domain, where they might directly regulate activity.

Unfortunately, only few of the DNMT1 PTMs have been functionally studied. The phosphorylated S515 in murine DNMT1 is involved in the interaction between the N-terminal and catalytic domains of DNMT1 and is necessary for the activity of the enzyme (96). Phosphorylation of S146 in the human enzyme introduced by casein kinase 1 delta/epsilon decreases the DNA binding affinity of DNMT1 (97), whereas phosphorylation of S127 and S143 regulates the interaction of DNMT1 with PCNA and UHRF1 (98). The S143 of DNMT1 is phosphorylated by AKT1, which leads to the stabilization of the methyltransferase (99). Recently, a specific 14-3-3 family reader protein for this modification was identified (100). It binds to the phosphorylated DNMT1, leading to the inhibition of DNMT1 activity *in vitro* and in cells, and subsequent aberrant DNA hypomethylation (100). Finally, phosphorylation of DNMT1 by protein kinase C (PKC) has been reported, but the target sites have not yet been identified (101). The functional significance of many other phosphorylations in DNMT1 still needs to be elucidated and, in particular, the influence of the PTMs on the allosteric regulation of DNMT1 activity, specificity and localization deserves further attention.

Multiple acetylation sites have been identified on DNMT1 up to date in proteomics analyses (102–104) (http://www.phosphosite.org); however, their functional significance has only begun to be revealed. Initial experiments with deacetylase inhibitors demonstrated the involvement of acetylation in the control of DNMT1 stability (104,105). Recently, an elegant mechanism regulating the abundance of DNMT1 during cell cycle has been identified. It starts with the acetylation of the lysine residues in the KG linker of DNMT1 catalyzed by the acetyltransferase Tip60 (77,79). KG acetylation leads to the UHRF1-mediated ubiquitination of DNMT1, resulting in its proteasomal degradation at the end of DNA replication. In turn, histone deacetylase 1 (HDAC1) increases the stability of DNMT1 (77,79). This effect is meditated by the deacetylation of the KG linker, followed by binding of the USP7 deubiquitinase to DNMT1, which prevents its ubiquitination (80). In addition, SIRT1 deacetylates DNMT1 at several sites and thereby regulates the activity and function of the methyltransferase as well (104).

The first described example of lysine methylation on DNMT1 was the monomethylation of K142 by SET7/9, which occurs mainly during late S-phase and promotes proteasomal degradation of the methyltransferase in a cell cycle-dependent manner (106). The methylation level is higher in the absence of the LSD1 lysine demethylase, suggesting that methylation of DNMT1 is reversible (107). In addition, as K142 is located directly next to S143, its methylation is antagonistic with the AKT1 mediated phosphorylation of S143 described above (99). This phospho-methyl switch is yet another example of the complex mechanisms that have evolved to fine-tune the function of DNMT1. We anticipate that many more examples of the crosstalk of DNMT1 PTMs will be discovered in the future. PTMs are ideally suited to mediate regulation of DNMT1 function, including either direct effects occurring through conformational changes of DNMT1 or by recruiting modification specific readers. Via both pathways, critical properties of the enzyme could be regulated, including enzyme stability, targeting, interaction with other proteins or its catalytic properties, like the overall activity, processivity and eventually specificity.

In summary, while we have already learned a lot about the structure and mechanism of DNMT1, there are still gaps in our understanding of its allosteric regulation, because all currently available structures of DNMT1 were obtained with truncated proteins, therefore, they might not represent the exact domain arrangement in the full-length enzyme. In addition, structures of posttranslationally modified DNMT1 are currently unavailable, and full-length DNMT1 structures in complex with interacting proteins are missing, raising the possibility that additional and perhaps even more complicated allosteric control effects might be discovered in the future. Moreover, as mentioned above, the thorough understanding of the atomic details of the recognition of the hemimethylated DNA awaits the availability of a structure containing an unmethylated substrate DNA and subsequent molecular dynamics simulations.

STRUCTURE, MECHANISM AND REGULATION OF DNMT3 ENZYMES

Domain composition of DNMT3 enzymes

The DNMT3 family comprises three paralogues, DNMT3A, DNMT3B and DNMT3L, which all share considerable sequence similarity (Figure 1). Several isoforms of DNMT3A and DNMT3B, resulting from alternative splicing or use of alternative start codons have been identified both in mouse and human cells (3). Like DNMT1, the DNMT3 proteins contain a C-terminal domain, which in the case of DNMT3A and DNMT3B contains the active center, and an N-terminal domain involved in the targeting of the enzymes to chromatin and regulation of their function (3). In the N-terminal part, two functional domains are present, the ADD (ATRX-DNMT3-DNMT3L) domain and the PWWP domain, which is missing in DNMT3L.

The ADD domain comprises three subdomains (108,109). The first subdomain contains four Cys residues, which bind a Zinc ion and form a GATA-like zinc finger. The second subdomain binds two zinc ions with eight Cys residues and forms a PHD finger with a cross-braced topology that is packed against the GATA-like finger. Finally, a long C-terminal α-helix that runs out from the PHD finger makes extensive hydrophobic contacts with the N-terminal GATA finger. The ADD domains mediate the interaction of all three DNMT3 proteins with histone H3 tails unmethylated at lysine K4 (110–113), similarly as in ATRX (114). In addition, this domain is involved in the interaction of DNMT3A with various components of the epigenetic machinery. Notably, the ADD domain has recently been implicated in the allosteric control of DNMT3A (see below), indicating that ADD-mediated interactions with other proteins could have direct regulatory effects on the catalytic activity of the MTase.

The PWWP domain of DNMT3A and DNMT3B is a conserved region of 100–150 amino acids, containing a characteristic proline–tryptophan motif (hence the name PWWP). PWWP domains belong to the Royal domain super-family, members of which interact with histone tails in various modification states (115). The structures of the PWWP domain from both DNMT3A and DNMT3B have been solved (116,117) and showed that it is divided into an N-terminal β-barrel, consisting of five antiparallel strands and a C-terminal helical bundle composed of five helices. The PWWP motif, which is a part of the β-barrel, is located at the interface of the two substructures (116). The PWWP domains of DNMT3A and DNMT3B specifically recognize the H3K36 trimethylation mark (118,119) and are essential for the targeting of the enzymes to pericentromeric chromatin (16,17). It has been suggested that the PWWP domain of DNMT3 enzymes would synergistically bind the H3K36me3 histone tail and the DNA through its conserved aromatic cage and a positively charged surface, respectively (115–117).

The part of DNMT3A and DNMT3B N-terminal to the PWWP domain is the least conserved region between both enzymes. Consequently, this part may be responsible for targeting of the enzymes to different genomic loci. This domain binds DNA (120) and is important for anchoring of

the enzymes to nucleosomes (18,119). However, up to date no specific molecular or biological function has been assigned to this part.

The C-terminal domains of all three DNMT3 proteins adopt the typical AdoMet-dependent MTase fold. The C-terminal domains of DNMT3A and DNMT3B share 81% sequence identity and contain the catalytic centers of the enzymes. The C-terminal domain of DNMT3L also shares clear homology with the other family members, but it contains several amino acid exchanges and deletions within the conserved DNA-(cytosine C5)-MTase motifs, indicating that DNMT3L cannot have catalytic activity and it is unable to bind AdoMet. In contrast to the catalytic domain of DNMT1, the catalytic domains of DNMT3A and DNMT3B are active in an isolated form (121) and have been used as model systems to study the catalytic mechanism and specificity of the DNMT3 proteins. Interestingly, the isolated catalytic domains of DNMT3A and DNMT3B show higher enzymatic activity than the full-length proteins, indicating that the N-terminal domains allosterically inhibit the activity of the enzymes (122), similarly as observed for DNMT1. Furthermore, kinetic experiments showed that binding of the ADD domain of DNMT3A to H3 tail peptides stimulates the activity of the enzyme (112–113,122). The molecular explanation for both these observations was provided recently by a seminal structural and biochemical study showing that the ADD domain of DNMT3A directly interacts with the catalytic domain of the methyltransferase and exerts an autoinhibitory function in the absence of histones (see below) (113) (Figure 5).

Tetrameric structure of the DNMT3A/DNMT3L C-terminal complex

Up to date, structures containing truncated DNMT3A–DNMT3L complexes (113,123) and one structure of full-length DNMT3L (110) have been solved. In addition, the structures of the isolated ADD and PWWP domains in a free and peptide-bound form are available (111,116–117). The structure of the complex of the C-terminal domains of DNMT3A/DNMT3L was solved in 2007, being the first published structure of a mammalian DNMT (123). It showed that the complex forms a linear heterotetramer consisting of two DNMT3L subunits (at the edges of the tetramer) and two DNMT3A subunits (in the center) (123) (Figure 5). The heterotetrameric structure of the complex was confirmed in solution (124). The structure revealed that the C-terminal domain of DNMT3A contains two interfaces for protein–protein contacts: a hydrophobic one generated by the stacking interaction of two phenylalanine residues (called FF interface), which mediates the DNMT3A/DNMT3L interaction, and a polar one stabilized by a hydrogen bonding network between arginine and aspartate residues from both subunits (called RD interface), which mediates DNMT3A/DNMT3A interaction. In contrast, DNMT3L contains only the FF interface since the region corresponding to the RD interface is absent. Residual electron density of the DNA ligand observed in the structure analysis and biochemical studies using interface mutants demonstrated that the central DNMT3A/DNMT3A interface creates the DNA binding site, while both interfaces are essential for AdoMet binding and catalytic activity (123,124). The dimerization of DNMT3A via the RD interface increases the size of the DNA interface and may therefore compensate for the small TRD of DNMT3A.

The spacing of the two active centers facing the DNA at the RD interface predisposes DNMT3A to optimally methylate CpG sites present in a distance of ∼10 bps. Indeed, *in vitro* methylation experiments demonstrated that there is a correlation of methylation between CpG sites localized ∼10 bps apart (123,124). Interestingly, an enrichment of CpG sites in such distance was observed in the differentially methylated regions (DMRs) of 12 maternally imprinted mouse genes, which are known biological substrates of the DNMT3A/DNMT3L complex, suggesting that the favorable CpG spacing could make these sequences good substrates for the MTase complex (123). Later, a 10 bps periodicity of methylation densities was observed in genome-wide methylation studies in human and plant DNA (91,125–128), suggesting that it represents a general pattern of genomic DNA methylation. Chodavarapu *et al*. (2010) proposed that this pattern might reflect limitations of the access to the DNA by nucleosomes due to the alternating exposure of the minor and major groove of the nucleosomal DNA with the same periodicity (127). However, none of these models to explain the 10 bp periodicity is flawless, because on one hand, the enzyme(s) potentially responsible for the 10 bps methylation periodicity in plants are not known, and on the other hand, the 10 bp periodicity was also observed outside of the nucleosome in the linker DNA. Hence, additional work is needed to further investigate these fundamental processes.

DNMT3A structures revealing allosteric control of its activity

Recently, new structures of a longer DNMT3A C-terminal fragment including the ADD domain in complex with the C-terminal domain of DNMT3L have been solved (113). They made an additional seminal contribution to our understanding of the mechanism of this enzyme by showing that the ADD domain can bind to the catalytic domain at two distinct sites, an allosteric one and an inhibitory one, creating two alternative conformations. ADD binding to the allosteric site keeps the enzyme active, while its binding to the inhibitory site blocks access of the DNA to the active center and inhibits catalysis (113) (Figure 5). These data indicate that the catalytic activity of DNMT3A, like that of DNMT1, is under allosteric control by rearrangements of its N-terminal domains, illustrating a fascinating convergence of the regulatory mechanisms of these two enzymes. Similarly as in DNMT1, protein partners and PMTs can influence the equilibrium of the active and inactive conformations. For example, the stimulatory effect of H3 on DNMT3A depends on its binding to the ADD domain, leading to the stabilization of the ADD at the allosteric binding site (see below) (113,122).

Specificity of DNMT3 enzymes

Consistent with their designation as *de novo* MTases, DNMT3A and DNMT3B do not discriminate between

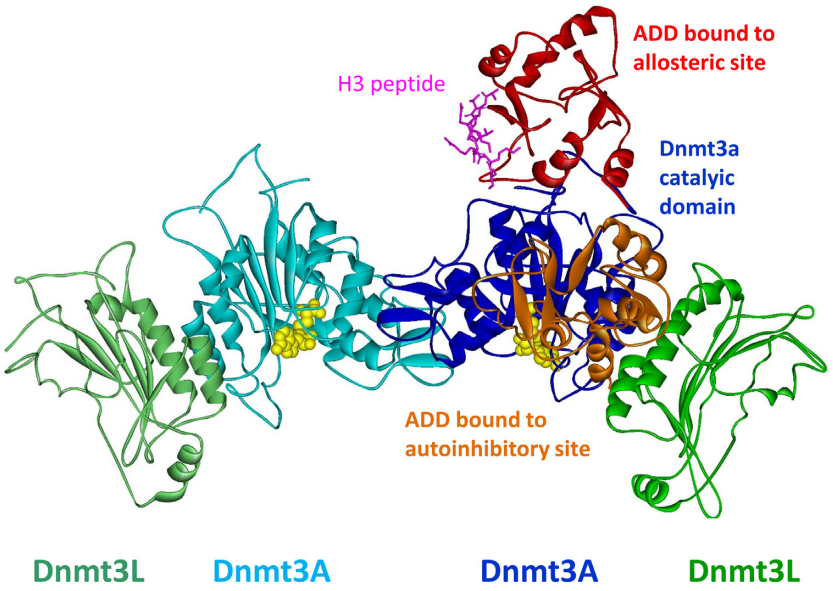

Figure 5. Structure and allosteric regulation of DNMT3A. The picture shows the structure of the DNMT3A/3L heterotetramer (123). The ADD domain of the dark blue DNMT3A subunit is shown in the autoinhibitory conformation (orange) and in the catalytically active allosteric conformation (red) (113), the ADD domain of the cyan DNMT3A subunit has been omitted for clarity. Binding of the H3 peptide (purple) to the ADD domain occurs by interaction with residues, which are involved in the autoinhibitory-binding interface. Therefore, peptide binding is only possible in the active conformation and this conformation is consequently stabilized in the presence of the H3 peptide (113,122).

hemimethylated and unmethylated DNA substrates. Although both enzymes methylate cytosine residues predominantly in the context of CpG dinucleotides, they can also introduce methylation in a non-CpG context with an apparent preference for CA >> CT > CC; and methylation rates at non-CpG sites can be as high as 50% of the rates at CpG sites (15,129–130). In contrast, the non-CpG methylation rates of DNMT1 are much lower (below 0.5% of what is observed with hemimethylated CpG sites) (49–50), indicating that the non-CpG methylation directly reflects the activity of DNMT3 enzymes. Consistently, methylated non-CpG sites are widespread in embryonic stem (ES) cells and brain, where DNMT3A and DNMT3B enzymes are highly expressed, but not in cells where DNMT3 enzymes are expressed only at low levels (126,131–133). However, a recent study revealed the presence of non-CpG methylation at lower levels in almost all human tissues (134), indicating that DNMT3 activity is indeed present in most tissues and it contributes to the overall DNA methylation patterns. Studies with different DNMT KO cell lines also confirmed that DNMT3 enzymes introduce non-CpG methylation in cells (135,136). A first glimpse into the biological role of non-CpG methylation was provided recently with the observation that non-CpG methylation can recruit MeCP2 (133,137–138), disruption of which is implicated in the Rett syndrome and that this process is involved in the repression of long genes in the brain (137).

In addition to their preference for the methylation of CpG sites, both DNMT3A and DNMT3B are very sensitive to the sequences flanking their target sites. This is illustrated by the finding that CpG sites embedded in certain flanking sequences cannot be methylated by DNMT3A at all (139). It has been shown that purine bases are preferred at the 5′ end of the CpG sites, whereas pyrimidines are favored at their 3′ end (139–141). Interestingly, experimental flanking sequence preferences of DNMT3A and DNMT3B correlate with the statistical data on the methylation level of CpG sites found in the human genome (91,141), suggesting that the inherent sequence preferences of *de novo* enzymes contribute to the selection of genomic regions that undergo methylation. One further consequence of the strong flanking sequence preferences of DNMT3A and DNMT3B is that both DNA strands of a CpG site, which are embedded in an asymmetric flanking sequence context, usually differ strongly in their ability to undergo methylation by DNMT3. This leads to the preferential methylation of one strand by the DNMT3 enzymes, generating hemimethylated products. Consistently, hemimethylated sites were detected in ES cells (136). *In vitro* experiments demonstrated that the products of DNMT3A methylation can be readily methylated by DNMT1 and both enzymes act synergistically to promote efficient *de novo* methylation of unmethylated DNA (82).

A deeper mechanistic understanding of the non-CpG recognition and flanking sequence preferences of DNMT3 enzymes awaits the availability of structures of DNMT3 enzymes with bound substrate DNA. An initial mutational analysis of residues in the (at that time putative) DNA binding site of DNMT3A showed that exchanges of critical residues pointing toward the DNA in the structural model caused massive changes of flanking sequence preferences of DNMT3A (32). These data confirmed that DNA binding and recognition is mediated by the cleft formed by the RD interface. Interestingly, the residues that potentially contact the DNA include R881, a residue frequently mutated in AML cancer (9), raising the possibility that this mutation might affect the DNA interaction of DNMT3A. In addition, some residues pointing toward the DNA binding cleft

differ between DNMT3A and DNMT3B, suggesting that DNMT3A and DNMT3B might differ in their specificity.

Mechanism of DNMT3 enzymes

Initial studies with the C-terminal domains of DNMT3A and DNMT3B showed an interesting difference in the catalytic mechanism of both enzymes. While DNMT3B methylated multiple CpG sites in a processive manner, DNMT3A was distributive, dissociating from DNA after each catalytic turnover (121). Later, it was reported that DNMT3A methylates DNA in a processive manner and that the processivity is increased by DNMT3L (142). However, DNMT3A also binds cooperatively to DNA, forming large multimeric protein/DNA fibers (123–124,143) (Figure 6B). These properties appear mutually exclusive, because the concept of a processive turnover is based on isolated enzyme complexes moving along a DNA substrate, which is not compatible with protein complexes multimerizing on DNA. Recent biochemical studies did not detect processive DNA methylation by DNMT3A (144).

Regulation of DNMT3 enzymes by protein oligomerization

DNMT3 enzymes exhibit a complex oligomerization and multimerization potential, including two independent orthogonal multimerization reactions (145). First, DNMT3A multimerizes on DNA and binds to it in a cooperative manner (horizontal polymerization), and secondly, DNMT3A can form protein oligomers able to bind to more than one DNA molecule (vertical polymerization) (Figure 6).

Multimerization of DNMT3A and DNMT3A/DNMT3L on DNA. As described above, DNMT3A forms a linear heterotetrameric complex with DNMT3L, in which two central DNMT3A subunits interacting via the RD interface generate the DNA binding site (123,124) (Figure 6A). DNA binding by DNMT3A is non-specific (143) and cooperative, as confirmed by different methods, including absence of binding intermediates in gel retardation assays and sigmoidal binding curves of DNA substrates in solution DNA binding experiments (123–124,143–144). The cooperative binding of DNMT3A (and DNMT3A/DNMT3L) complexes next to each other on DNA leads to the formation of DNMT3–DNA filaments observed by atomic force microscopy imaging (124,143) (Figure 6B). Interestingly, two adjacent DNMT3 complexes in such filament can contact the cytosine residues in the upper and lower DNA strand of the same CpG site, providing an option for the enzyme to directly methylate one CpG site in both DNA strands (Figure 6C). The 10 bps spacing between the two active centers within the DNMT3 complexes leads to a characteristic profile of DNA methylation introduced by the DNMT3A multimers on DNA, with peaks observed at distances of 8–10 bps (124).

The interface of adjacent DNMT3A complexes bound to DNA was mapped to a loop within the putative TRD of DNMT3A, and mutation of residues within this loop disrupted multimerization (143). Interestingly, it also led to the loss of heterochromatic enrichment of DNMT3A, suggesting that cooperative DNA binding and multimerization of DNMT3A complexes on DNA contribute to the heterochromatic localization of the enzyme in cells. Furthermore, recent studies have shown that the cooperative binding of DNMT3A to long DNA substrates increases the rate of DNA methylation *in vitro* (144) and in targeted DNA methylation experiments in cells (Stepper *et al*., manuscript submitted for publication), indicating that it is important for DNA methylation by DNMT3A.

Cooperative DNA binding of DNMT3A increases its DNA binding affinity and reduces the rate of dissociation, which might help anchoring the MTase on DNA, providing a molecular explanation for its strong binding to methylated chromatin (18,19). However, the exact role of the cooperative DNA binding of DNMT3A in live cells needs further investigation and the sizes of DNMT3A–DNA filaments in cells are currently unknown. Based on the crystal structure, one may speculate that binding of up to five DNMT3 protein complexes would be possible in the linker DNA regions of chromatin. This is in agreement with biochemical data showing preferential methylation of linker DNA by DNMT3 enzymes *in vitro* (146–148). Similarly, *in vivo* studies showed that DNMT3B expressed in yeast preferentially methylates linker DNA (149) and an analogous methylation pattern was observed after reintroduction of the DNMT3 enzymes into DNMT3 KO ES cell lines (119). Interestingly, genome-wide methylation studies revealed that DNA bound to nucleosomes is higher methylated on average than linker DNA (127). However, in this study methylation levels reflected the steady-state of both the methylation and demethylation activities, and it is very plausible that nucleosome occupancy also reduces the accessibility of the DNA for DNA demethylation processes. In contrast, in the yeast study, DNA demethylation activity is absent, therefore, the observed methylation pattern directly reflects the DNMT activity.

Longer DNMT3 filaments might form if DNMT3 binding is coupled to nucleosome remodeling. Consistently, DNMT3 enzymes form complexes with various chromatin remodelers, including SMARCA4 (150), CHD4 (151), hSNF2 (152) and HELLS (153,154). Notably, the interaction with HELLS is essential for *de novo* DNA methylation (155,156). In line with this model, remodeling activity promotes the methylation of nucleosomal DNA (148).

Protein multimerization of DNMT3 enzymes. Aside its ability to form heterotetrameric complexes with DNMT3L and to polymerize on DNA, DNMT3A alone can also form protein filaments (Figure 6D), which can lead to its reversible aggregation, as observed in different studies (157,158). This is possible, because the FF interface of the DNMT3A/DNMT3L tetramer is symmetric, so that it also supports the interaction of two DNMT3A molecules in addition to the interaction of DNMT3A with DNMT3L. Hence, each DNMT3A subunit contains two interfaces for self-interactions, the RD interface and the FF interface and the alternative use of both interfaces leads to the formation of linear DNMT3A protein fibers. Notably, addition of DNMT3L directs the preferential formation of defined DNMT3A/DNMT3L heterotetramers that cannot oligomerize further, because DNMT3L does not contain an RD interface and therefore functions as a cap in DNMT3

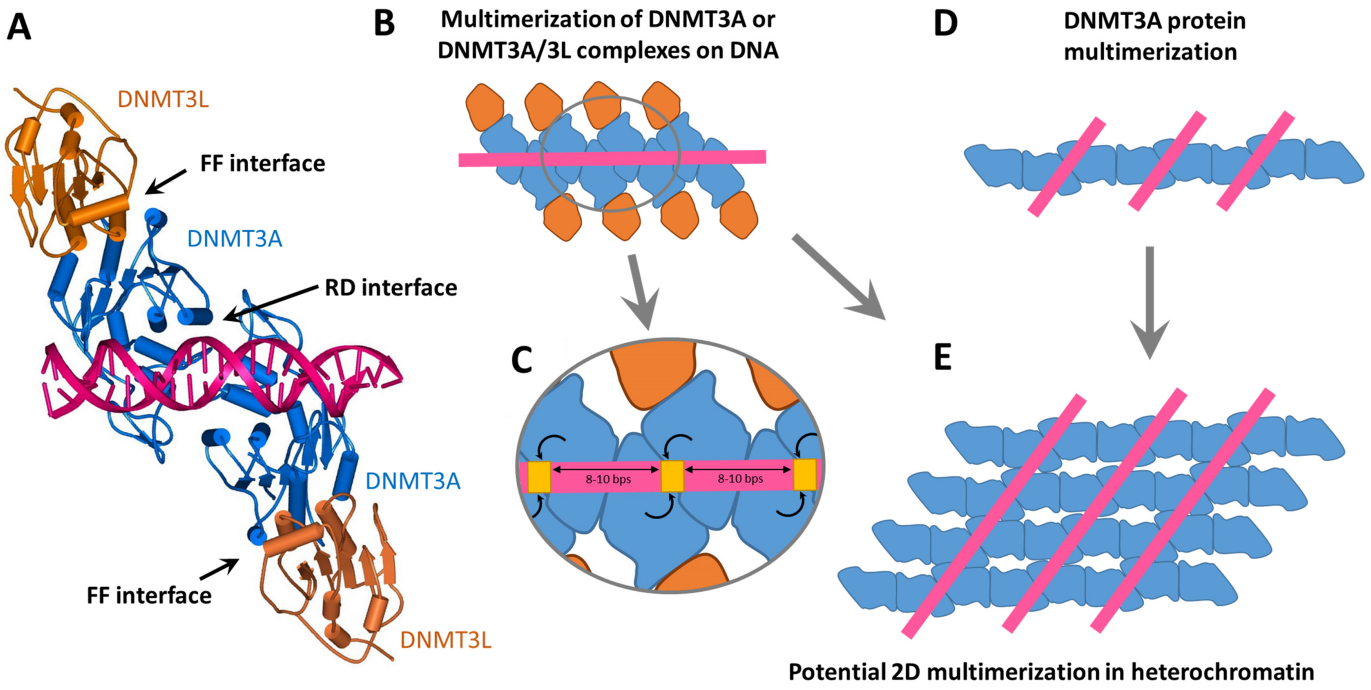

Figure 6. Multimerization of DNMT3A and DNMT3A/DNMT3L complexes. (**A**) Structure of the DNMT3A–DNMT3L complex with bound DNA. (**B**) Schematic model of multimerization of the DNMT3A/DNMT3L complexes on DNA. (**C**) Magnification of a part of B showing the access of active centers of the central DNMT3A subunits to both DNA strands of CpG sites (yellow boxes). (**D**) Schematic model of DNMT3A protein multimerization and binding to several DNA molecules. (**E**) Combination of both multimerization processes leading to the 2D multimerization of DNMT3A.

protein multimerization. As described below, this process is implicated in the release of DNMT3A from heterochromatic sites (157).

Since each RD interface of an DNMT3A oligomer constitutes a potential DNA binding site, the protein oligomers can bind to more than one DNA molecule, provided that they are oriented roughly in parallel (157) (Figure 6D), and a combination of both multimerization reactions could lead to the formation of two-dimensional (2D) DNMT3–DNA networks (Figure 6E). The ability to form protein oligomers plays a central role in the heterochromatin localization of DNMT3A, as non-oligomerizing DNMT3A mutants affected at any of the two interfaces lost the ability to bind to heterochromatin, despite the presence of intact PWWP and ADD domains. Since heterochromatic DNA is densely packed, it might provide several DNA strands for DNMT3A interaction in matching geometry, contributing to anchoring of DNMT3A to pericentromeric chromatin.

Despite significant progress in dissecting protein multimerization of the DNMT3 enzymes, many questions still await elucidation. For example, based on the sequence similarity and the interface conservation, DNMT3B is expected to self-oligomerize as well, however, the oligomeric state of DNMT3B is unknown at present. Similarly, DNMT3A forms catalytically active heterodimers with DNMT3B that use the same interfaces as described above for DNMT3A (159). However, the relative affinities for the self-interactions of DNMT3A and DNMT3B, as compared to the interaction of DNMT3A with DNMT3B at the two interfaces are unknown. Equally, the relative preferences of DNMT3A and DNMT3B for binding DNMT3L at the FF interface have not been determined, although the preferential formation of defined heterotetramers of DNMT3A and DNMT3L suggests that at the FF interface the DNMT3A/DNMT3L interaction is stronger than the self-interaction of DNMT3A. Finally, the direct proof for the existence of DNMT3 protein multimers in cells that are larger than the tetrameric structure observed in the DNMT3A/DNMT3L complex still needs to be provided.

Targeting and regulation of DNMT3 enzymes by direct chromatin interaction

The ADD and the PWWP domains located in the N-terminal part of DNMT3 proteins were shown to target the MTases to chromatin carrying defined modifications. When combined with the DNA binding through DNMT3 multimerization, a multivalent interaction of the DNMT3 enzymes with chromatin occurs, which may explain the extraordinarily strong binding of these enzymes to nucleosomal heterochromatic DNA (18,19).

Binding of the DNMT3 ADD domain to H3 tails. In 2007, binding of the H3 tails to the ADD domain of DNMT3L was described, a seminal discovery showing for the first time the direct targeting of a DNMT to chromatin via histone tail interactions (110). Today, it is clear that the ADD domains of all three DNMT3 proteins interact specifically with histone H3 tails (Figure 5), and that the binding is disrupted by any larger modifications of K4 (di- and trimethylation or acetylation), phosphorylation of T6 or acetylation of the H3 N-terminus (110–112,160). Inter-

estingly, H3K4me1, which is observed at enhancers, hinders the binding of the ADD domain less strongly than di- or trimethylation (112,160), however the functional consequences of this difference are unknown. The structures of the ADD domains from DNMT3A and DNMT3L in complex with histone H3 tail peptides were solved (110–111,113). They showed several hydrogen bonding contacts to both the N-terminal and lysine 4 side chain amino groups that explain the specific recognition of the H3 peptide.

Binding to H3 tails directly activates DNMT3A by an allosteric mechanism (122) and stimulates the methylation of chromatin-bound DNA by DNMT3A *in vitro* (112,113). These results indicate that the ADD domain of DNMT3A guides DNA methylation in response to specific histone modifications and provide strong evidence that DNA methyltransferases could be targeted to chromatin carrying specific marks. Indeed, a strong correlation of DNA methylation with the absence of H3K4me3 that was observed in several genome-wide studies (90–91,161–162) suggests that this mechanism plays an important role in the generation of the genomic DNA methylation pattern. This hypothesis was recently experimentally verified, as DNMT3B artificially introduced into yeast did not methylate genomic regions with high H3K4me3 content (149) and a DNMT3A enzyme with an engineered ADD domain able to tolerate K4 methylation or T6 phosphorylation generated abnormal DNA methylation patterns in cells (160).

The mechanism of the regulation of DNMT3A activity by the ADD domain has recently been elucidated in a seminal structural analysis by Guo *et al.*, which showed that unexpectedly the ADD domain could bind to the catalytic domain of DNMT3A at two alternative sites, an allosteric site and an autoinhibitory site, in which the ADD domain blocks the access of DNA to the DNA binding cleft, leading to enzyme inhibition (113). Different ADD residues contact the two regions of the catalytic domain in the two conformations. Strikingly, critical residues contacting the catalytic domain in the autoinhibitory conformation (like D529 and D531) are also involved in direct interactions with the H3 peptide (113). Binding of the H3 peptide to the ADD domain consequently favors the allosteric conformation and activates DNMT3A (112–113,122). In extrapolation, it is very likely that other DNMT3A interaction partners (particularly if the binding occurs via the ADD domain) and PTMs can affect the conformation of DNMT3A in a similar manner and thereby directly regulate the catalytic activity of the enzyme. Moreover, given the fact that the DNMT3 enzymes contain additional N-terminal domains, which would also be rearranged if the ADD domain changes its binding site, it is quite conceivable that the allosteric control also affects the multimerization and eventually processivity of DNMT3 enzymes.

Binding of the DNMT3 PWWP domain to H3 tails methylated at K36. The PWWP domain is essential for the targeting of DNMT3A and DNMT3B to pericentromeric chromatin (16,17) and to gene bodies via specific recognition of histone H3 tails trimethylated at lysine 36 (118). This finding explains the genome-wide correlation of DNA methylation and H3K36me3 methylation, both in gene bodies, as well as in heterochromatin. H3K36me3 accumulates in euchromatin in the body of active genes and its distribution is anti-correlated with H3K4me3 (163–167). DNA methylation of gene bodies mirrors that pattern, with gene bodies of active genes showing high and those of inactive genes low methylation (168,169). A correlation of H3K36me3 and DNA methylation was also observed at exon–intron boundaries, with exons showing increased levels of both H3K36me3 (170) and DNA methylation (161). In addition, a subset of heterochromatin containing repetitive sequences with copy number variations is strongly enriched in H3K36me3 (171), illustrating that direct recognition of this mark by the PWWP domain of DNMT3A contributes to the heterochromatic localization of the enzyme. The combined targeting of DNMT3A and DNMT3B by their ADD and PWWP domains explains the strong correlation of DNA methylation with the absence of H3K4me3 and presence of H3K36me3 observed in genome-wide DNA methylation studies (90,161).

The central role of K36 methylation in the targeting of DNMT3 enzymes has been lately experimentally confirmed in yeast (149). In addition, a recent study showed that the methylation of gene bodies by DNMT3B in embryonic cells directly depends on both the H3K36 methylation and an intact PWWP domain (119). Interestingly, the interaction of DNMT3A with H3K36me3 increases the activity of DNMT3A on modified chromatin in *in vitro* (118), suggesting that binding to H3 tails methylated at K36 might have effects beyond the targeting of the DNMT3 enzymes. However, whether there exists an allosteric mechanism connecting the PWWP domain with the catalytic domain, similarly as observed for the ADD domain, remains currently unknown.

In addition to H3 peptide binding, the PWWP domains of DNMT3A and DNMT3B can interact with DNA, with DNMT3B PWWP binding DNA more strongly (116,172). Moreover, a combined interaction with methylated H3K36 and DNA has been observed for other PWWP domains as well (118,173). This finding is not unexpected, as the K36 side chain emerges from the nucleosome body next to the bound DNA. Simultaneous H3K36me3 and DNA binding by PWWP domains are mediated by two adjacent interfaces, one featuring an aromatic cage for peptide binding and the other one presenting a basic region for DNA interaction. Recently, a mechanism for the recognition of nucleosomes and subsequent DNA methylation by DNMT3A was proposed, based on a structural model of the full-length DNMT3A/DNMT3L heterotetramer in complex with an H3K36me3-modified dinucleosome (117). It suggested that the targeting of DNMT3A occurs through a specific recognition and binding of H3K36me3 and DNA by the PWWP domain, which is followed by an activation of the catalytic domain through the binding of H3 tails unmodified at K4 to the ADD domain, resulting in the methylation of nearby cytosines by the catalytic domain. In addition, this model incorporates that DNA methylation by the heterotetramer preferentially occurs on the linker DNA between the nucleosomes, which is in agreement with the results of *in vitro* and *in vivo* studies, as described above (119,146–149).

Regulation of DNMT3 enzymes by interacting proteins

Many proteins with important roles in chromatin biology were found to interact with DNMT3A though the ADD domain, including protein lysine methyltransferases SUV39H1 (174), SETDB1 (175) and E2H2 (176), deacetylase HDAC1, reading domain proteins, including HP1-beta (174), Mbd3 (150) and MeCP2 (177,178) (Rajavelu *et al*., manuscript in preparation), as well as transcriptional factors PU.1 (179), Myc (180) and RP58 (181) and remodeling factors hSNF2 (152) and SMARCA4 (150). In addition, the ZHX1 (zinc-finger and homeobox protein 1) interacts with the PWWP domain of DNMT3B and enhances DNMT3B-mediated transcriptional repression (182) and HP1alpha recruits DNMT3B to major satellite repeats at pericentric heterochromatin, contributing to their methylation (183). However, up to date, only the interaction of DNMT3 enzymes with DNMT3L has been studied in greater mechanistic details, revealing important roles for DNMT3L in targeting, allosteric regulation and control of multimerization of the *de novo* MTases. Notably, in light of the emerging concept of DNMT3 regulation by domain rearrangements, the numerous additional interactors could have a direct impact not only on the targeting, but also on the activity of the methyltransferases. Therefore, in order to understand the regulation of DNMT3 enzymes in cells, further efforts delineating the molecular mechanism of these interactions are urgently needed. In particular, direct effects on the catalytic activity via allosteric mechanisms should be investigated.

DNMT3A–DNMT3L interaction. DNMT3L co-localizes with both DNMT3A and DNMT3B in mammalian cells (184). It directly interacts with its C-terminal domain with the catalytic domains of DNMT3A and DNMT3B and stimulates the activity of both enzymes *in vivo* (185,186) and *in vitro* (158,187–188). DNMT3L is expressed during gametogenesis and embryonic stages, where it functions as a stimulatory factor of DNTM3A and is needed for the establishment of DNA methylation patterns in the developing germ line cells (184,189–190). The structure of the complex of the C-terminal domains of DNMT3A and DNMT3L provided the mechanistic explanation for the observed stimulatory effect of DNMT3L by revealing that the interaction of both proteins through the FF interface influences the structure of DNMT3A via the α-helices C, D and E. Residues from these helices directly interact with the key catalytic and AdoMet binding residues, which may explain the positive effect DNMT3L exerts on DNMT3A AdoMet binding and catalysis (123).

As described above, binding of DNMT3L to DNMT3A leads to the disruption of DNMT3A protein oligomers. Importantly, it also changes the sub-nuclear localization of DNMT3A in cells (Figure 7). *In vivo*, DNMT3L was shown to release DNMT3A from heterochromatin, by converting large DNMT3A oligomers into defined tetramers, which are homogenously distributed in the cell nucleus (157). The redistribution of DNMT3A may be important for the methylation of imprinted differentially methylated regions (DMRs) and other targets in gene promoters, which generally are euchromatic. This finding goes in line with the discovery that DNMT3L favors DNA methylation in gene bodies (191). Hence, DNMT3L, which was originally discovered as a stimulator of DNMT3A (188), also changes the sub-nuclear localization of this enzyme (157), providing the first example of a protein partner that influences multiple aspects of DNMT3A function. Recent data indicate that the combined regulation of activity and localization of DNMT3A also applies to other regulatory cues (see below for the CK2-mediated phosphorylation of DNMT3A) and might be a general mechanism of regulation for this family of enzymes (Figures 3 and 5).

Interestingly, recently an investigation of the function of catalytically inactive DNMT3B isoforms has shown that these proteins stimulate DNA methylation by DNMT3B (192). The observation that DNMT3L is predominantly expressed in undifferentiated cells while the DNMT3B isoforms are expressed in differentiated cells and also in tumor cells brought the authors to the proposal that the DNMT3B isoforms can functionally substitute DNMT3L in these cells. The molecular mechanism of the stimulation has not yet been clarified in detail.

Regulation of DNMT3 enzymes by nucleic acid binding

Similar as in DNMT1, additional DNA binding sites have been identified in the N-terminal parts of the DNMT3 enzymes, but their exact role in DNMT3 function remains unknown. As described above, the isolated PWWP domain of DNMT3B features a DNA binding activity (116) and an additional DNA binding site was detected in the PWWP domain of DNMT3A as well (172). Equally, the very N-terminal part of DNMT3A is able to bind DNA (120). Moreover, it was shown that long non-coding RNAs bind strongly to the catalytic domain of DNMT3A, causing inhibition of the enzyme (193). The authors also detected binding of RNA to allosteric sites that did not change the catalytic activity. Besides, it was shown that a non-coding RNA derived from the rDNA promoter binds to the promoter forming RNA/DNA triplex structures that are specifically recognized by DNMT3B, establishing a novel pathway of RNA-directed DNA methylation (194,195). Future work will show if RNA triplex-based recruitment emerges as new and general principle of the RNA-dependent recruitment of DNMTs and other chromatin interacting enzymes.

Regulation of DNMT3A by phosphorylation

The regulation of the DNMT3 enzymes by phosphorylation has not been studied almost at all, despite the fact that more than 70 phosphorylation sites have been identified in DNMT3A and DNMT3B in global proteomics studies (http://www.phosphosite.org). So far, only one PTM of DNMT3 enzymes has been studied in greater mechanistic depth (196). Casein kinase 2 (CK2), a so-called survival protein kinase that suppresses cancer cell death and is often upregulated in cancers, was shown to phosphorylate DNMT3A at two sites; S386 and S389, which are located next to the PWWP domain. The CK2-mediated phosphorylation increases the heterochromatic targeting of DNMT3A and reduces its DNA methylation activity. This effect was reflected by changes in the cellular DNA methylation after CK2 knockout, which may explain global hypomethylation

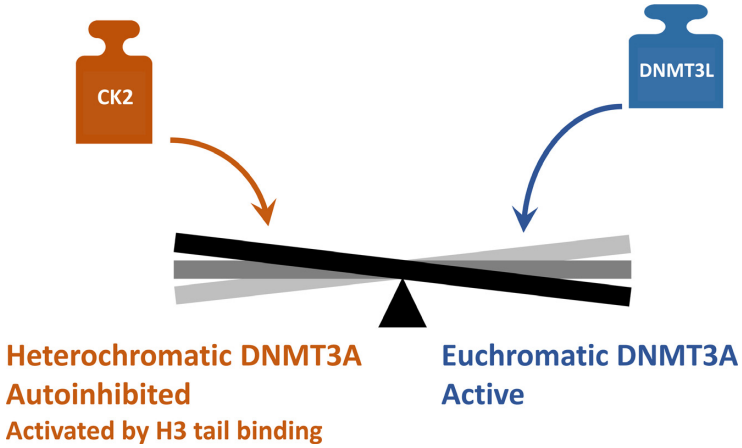

Figure 7. Mechanisms regulating the activity and localization of DNMT3A. Interactors and PTMs regulate the activity and localization in a concerted fashion. DNMT3L stimulates DNMT3A and promotes its euchromatic localization. Contrarily, CK2-mediated phosphorylation downregulates the activity of DNMT3A and promotes its heterochromatic localization, where the interaction with modified H3 tails could allosterically stimulate the enzyme.

in cancer cells overexpressing CK2. These data nicely support our model that the combined regulation of enzymatic activity and localization is a general principle in the regulation of DNMT3A (as already observed for DNMT3L interaction) (Figure 7). Whether allosteric processes mediate this regulation is not known at present.

CONCLUSIONS AND OUTLOOK

After almost 40 years of intensive research in the DNA methylation field, we have learned a great deal about the biochemical, structural and enzymatic properties of the mammalian DNA methyltransferases. However, the regulation of these fascinating enzymes in cells has only begun to be uncovered. Importantly, it has been lately realized that the precise control of DNMT activity is critically involved in the generation and maintenance of the dynamic DNA methylation patterns in living cells. Recent crystallographic studies with DNMT1 and DNMT3A revealed that both enzymes unexpectedly undergo large domain rearrangements, which allosterically regulate their catalytic activity. This unforeseen discovery has led to the important conclusion that by influencing domain rearrangements, any PTMs or interaction partner (be it a protein, an allosteric DNA or a non-coding RNA) at various parts of the methyltransferases could directly regulate the enzymatic activity and specificity of the DNMTs via allosteric effects, providing new and fascinating perspectives on the investigation of the effects of interactors and PTMs on these enzymes.

The combined regulation of targeting of DNMTs with the regulation of their activity strongly increases the efficiency of DNMT control, because the enzymes can be kept inactive until they are delivered to their target regions and only become activated at these sites. The need for a very tight control of the activity of critical enzymes might explain, why similar allosteric regulation mechanisms evolved for several other epigenetic enzymes as well, including the MLL, SUV39H1 and PRC2 histone methyltransferases, which deliver the H3K4, K9 and K27 methylation marks, respectively (197–199).

Finally, non-coding RNAs have been recognized as an emerging player in chromatin regulation (200,201) and RNA molecules were shown to influence DNA methylation. In plants, a process of RNA-dependent DNA methylation exists, in which the RNA sequence directly guides DNA methylation (202). Although this pathway is absent in mammals, binding of small and long non-coding RNAs to mammalian DNMTs was shown to guide and regulate their activity. In addition, the piRNA-mediated DNA methylation in the germ line of many animals, including mammals (203), recapitulates many features of an RNA-directed DNA methylation pathway, but it is not yet understood at molecular level. The direct targeting and regulation of DNA methylation by genome-encoded non-coding RNAs adds another dimension to the complex interplay between genetic information (encoded in the DNA sequence) and epigenetic information (encoded in the chromatin modification pattern, including DNA methylation).

FUNDING

Work in the authors laboratory has been supported by the DFG [JE252/10-1] and the Carl Zeiss foundation. Funding for open access charge were provided by Stuttgart University.
Conflict of interest statement. None declared.

REFERENCES

1. Jeltsch,A. and Jurkowska,R.Z. (2014) New concepts in DNA methylation. *Trends Biochem. Sci.*, **39**, 310–318.
2. Jones,P.A. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.*, **13**, 484–492.
3. Jurkowska,R.Z., Jurkowski,T.P. and Jeltsch,A. (2011) Structure and function of mammalian DNA methyltransferases. *Chembiochem*, **12**, 206–222.
4. Klose,R.J. and Bird,A.P. (2006) Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.*, **31**, 89–97.
5. Suzuki,M.M. and Bird,A. (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nat. Rev. Genet.*, **9**, 465–476.
6. Baylin,S.B. and Jones,P.A. (2011) A decade of exploring the cancer epigenome - biological and translational implications. *Nat. Rev. Cancer*, **11**, 726–734.

7. Bergman,Y. and Cedar,H. (2013) DNA methylation dynamics in health and disease. *Nat. Struct. Mol. Biol.*, **20**, 274–281.
8. Suva,M.L., Riggi,N. and Bernstein,B.E. (2013) Epigenetic reprogramming in cancer. *Science*, **339**, 1567–1570.
9. Hamidi,T., Singh,A.K. and Chen,T. (2015) Genetic alterations of DNA methylation machinery in human diseases. *Epigenomics*, **7**, 247–265.
10. Wu,T.P., Wang,T., Seetin,M.G., Lai,Y., Zhu,S., Lin,K., Liu,Y., Byrum,S.D., Mackintosh,S.G., Zhong,M. *et al.* (2016) DNA methylation on N(6)-adenine in mammalian embryonic stem cells. *Nature*, **532**, 329–333.
11. Luo,G.Z., Blanco,M.A., Greer,E.L., He,C. and Shi,Y. (2015) DNA N(6)-methyladenine: A new epigenetic mark in eukaryotes? *Nat. Rev. Mol. Cell Biol.*, **16**, 705–710.
12. Zhang,G., Huang,H., Liu,D., Cheng,Y., Liu,X., Zhang,W., Yin,R., Zhang,D., Zhang,P., Liu,J. *et al.* (2015) N6-methyladenine DNA modification in Drosophila. *Cell*, **161**, 893–906.
13. Greer,E.L., Blanco,M.A., Gu,L., Sendinc,E., Liu,J., Aristizabal-Corrales,D., Hsu,C.H., Aravind,L., He,C. and Shi,Y. (2015) DNA Methylation on N6-Adenine in C. elegans. *Cell*, **161**, 868–878.
14. Okano,M., Xie,S.P. and Li,E. (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat. Genet.*, **19**, 219–220.
15. Gowher,H. and Jeltsch,A. (2001) Enzymatic properties of recombinant Dnmt3a DNA methyltransferase from mouse: The enzyme modifies DNA in a non-processive manner and also methylates non-CpA sites. *J. Mol. Biol.*, **309**, 1201–1208.
16. Chen,T.P., Tsujimoto,N. and Li,E. (2004) The PWWP domain of Dnmt3a and Dnmt3b is required for directing DNA methylation to the major satellite repeats at pericentric heterochromatin. *Mol. Cell. Biol.*, **24**, 9048–9058.
17. Ge,Y.Z., Pu,M.T., Gowher,H., Wu,H.P., Ding,J.P., Jeltsch,A. and Xu,G.L. (2004) Chromatin targeting of de novo DNA methyltransferases by the PWWP domain. *J. Biol. Chem.*, **279**, 25447–25454.
18. Jeong,S., Liang,G.N., Sharma,S., Lin,J.C., Choi,S.H., Han,H., Yoo,C.B., Egger,G., Yang,A.S. and Jones,P.A. (2009) Selective Anchoring of DNA Methyltransferases 3A and 3B to Nucleosomes Containing Methylated DNA. *Mol. Cell. Biol.*, **29**, 5366–5376.
19. Sharma,S., De Carvalho,D.D., Jeong,S., Jones,P.A. and Liang,G. (2011) Nucleosomes containing methylated DNA stabilize DNA methyltransferases 3A/3B and ensure faithful epigenetic inheritance. *PLoS Genet.*, **7**, e1001286.
20. Jeltsch,A. (2002) Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases. *Chembiochem*, **3**, 275–293.
21. Hermann,A., Gowher,H. and Jeltsch,A. (2004) Biochemistry and biology of mammalian DNA methyltransferases. *Cell. Mol. Life Sci.*, **61**, 2571–2587.
22. Cheng,X. and Blumenthal,R.M. (2008) Mammalian DNA methyltransferases: A structural perspective. *Structure*, **16**, 341–350.
23. Robertson,K.D., Uzvolgyi,E., Liang,G., Talmadge,C., Sumegi,J., Gonzales,F.A. and Jones,P.A. (1999) The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. *Nucleic Acids Res.*, **27**, 2291–2298.
24. Easwaran,H.P., Schermelleh,L., Leonhardt,H. and Cardoso,M.C. (2004) Replication-independent chromatin loading of Dnmt1 during G2 and M phases. *EMBO Rep.*, **5**, 1181–1186.
25. Leonhardt,H., Page,A.W., Weier,H.U. and Bestor,T.H. (1992) A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell*, **71**, 865–873.
26. Riggs,A.D. and Xiong,Z. (2004) Methylation and epigenetic fidelity. *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 4–5.
27. Jones,P.A. and Liang,G. (2009) Rethinking how DNA methylation patterns are maintained. *Nat. Rev. Genet.*, **10**, 805–811.
28. Cheng,X. and Roberts,R.J. (2001) AdoMet-dependent methylation, DNA methyltransferases and base flipping. *Nucleic Acids Res.*, **29**, 3784–3795.
29. Song,J., Teplova,M., Ishibe-Murakami,S. and Patel,D.J. (2012) Structure-based mechanistic insights into DNMT1-mediated maintenance DNA methylation. *Science*, **335**, 709–712.
30. Du,Q., Wang,Z. and Schramm,V.L. (2016) Human DNMT1 transition state structure.*Proc. Natl. Acad. Sci. U.S.A.*, **113**, 2916–2921.
31. Reither,S., Li,F., Gowher,H. and Jeltsch,A. (2003) Catalytic mechanism of DNA-(cytosine-C5)-methyltransferases revisited: covalent intermediate formation is not essential for methyl group transfer by the murine Dnmt3a enzyme. *J. Mol. Biol.*, **329**, 675–684.
32. Gowher,H., Loutchanwoot,P., Vorobjeva,O., Handa,V., Jurkowska,R.Z., Jurkowski,T.P. and Jeltsch,A. (2006) Mutational analysis of the catalytic domain of the murine Dnmt3a DNA-(cytosine C5)-methyltransferase. *J. Mol. Biol.*, **357**, 928–941.
33. Lukashevich,O.V., Cherepanova,N.A., Jurkovska,R.Z., Jeltsch,A. and Gromova,E.S. (2016) Conserved motif VIII of murine DNA methyltransferase Dnmt3a is essential for methylation activity. *BMC Biochem.*, **17**, 7.
34. Rountree,M.R., Bachman,K.E. and Baylin,S.B. (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat. Genet.*, **25**, 269–277.
35. Mohan,K.N., Ding,F. and Chaillet,J.R. (2011) Distinct roles of DMAP1 in mouse development. *Mol. Cell. Biol.*, **31**, 1861–1869.
36. Ding,F. and Chaillet,J.R. (2002) In vivo stabilization of the Dnmt1 (cytosine-5)- methyltransferase protein. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 14861–14866.
37. Chuang,L.S., Ian,H.I., Koh,T.W., Ng,H.H., Xu,G. and Li,B.F. (1997) Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science*, **277**, 1996–2000.
38. Egger,G., Jeong,S., Escobar,S.G., Cortez,C.C., Li,T.W., Saito,Y., Yoo,C.B., Jones,P.A. and Liang,G. (2006) Identification of DNMT1 (DNA methyltransferase 1) hypomorphs in somatic knockouts suggests an essential role for DNMT1 in cell survival. *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 14080–14085.
39. Suetake,I., Hayata,D. and Tajima,S. (2006) The amino-terminus of mouse DNA methyltransferase 1 forms an independent domain and binds to DNA with the sequence involving PCNA binding motif. *J. Biochem.*, **140**, 763–776.
40. Berkyurek,A.C., Suetake,I., Arita,K., Takeshita,K., Nakagawa,A., Shirakawa,M. and Tajima,S. (2014) The DNA methyltransferase Dnmt1 directly interacts with the SET and RING finger-associated (SRA) domain of the multifunctional protein Uhrf1 to facilitate accession of the catalytic center to hemi-methylated DNA. *J. Biol. Chem.*, **289**, 379–386.
41. Bashtrykov,P., Jankevicius,G., Jurkowska,R.Z., Ragozin,S. and Jeltsch,A. (2014) The UHRF1 protein stimulates the activity and specificity of the maintenance DNA methyltransferase DNMT1 by an allosteric mechanism. *J. Biol. Chem.*, **289**, 4106–4115.
42. Pradhan,M., Esteve,P.O., Chin,H.G., Samaranayke,M., Kim,G.D. and Pradhan,S. (2008) CXXC domain of human DNMT1 is essential for enzymatic activity. *Biochemistry*, **47**, 10000–10009.
43. Song,J., Rechkoblit,O., Bestor,T.H. and Patel,D.J. (2011) Structure of DNMT1-DNA complex reveals a role for autoinhibition in maintenance DNA methylation. *Science*, **331**, 1036–1040.
44. Bashtrykov,P., Jankevicius,G., Smarandache,A., Jurkowska,R.Z., Ragozin,S. and Jeltsch,A. (2012) Specificity of Dnmt1 for methylation of hemimethylated CpG sites resides in its catalytic domain. *Chem. Biol.*, **19**, 572–578.
45. Du,J., Zhong,X., Bernatavichute,Y.V., Stroud,H., Feng,S., Caro,E., Vashisht,A.A., Terragni,J., Chin,H.G., Tu,A. *et al.* (2012) Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants. *Cell*, **151**, 167–180.
46. Takeshita,K., Suetake,I., Yamashita,E., Suga,M., Narita,H., Nakagawa,A. and Tajima,S. (2011) Structural insight into maintenance methylation by mouse DNA methyltransferase 1 (Dnmt1). *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 9055–9059.
47. Syeda,F., Fagan,R.L., Wean,M., Avvakumov,G.V., Walker,J.R., Xue,S., Dhe-Paganon,S. and Brenner,C. (2011) The replication focus targeting sequence (RFTS) domain is a DNA-competitive inhibitor of Dnmt1. *J. Biol. Chem.*, **286**, 15344–15351.
48. Zhang,Z.M., Liu,S., Lin,K., Luo,Y., Perry,J.J., Wang,Y. and Song,J. (2015) Crystal Structure of Human DNA Methyltransferase 1. *J. Mol. Biol.*, **427**, 2520–2531.
49. Bashtrykov,P., Ragozin,S. and Jeltsch,A. (2012) Mechanistic details of the DNA recognition by the Dnmt1 DNA methyltransferase. *FEBS Lett.*, **586**, 1821–1823.

50. Fatemi,M., Hermann,A., Pradhan,S. and Jeltsch,A. (2001) The activity of the murine DNA methyltransferase Dnmt1 is controlled by interaction of the catalytic domain with the N-terminal part of the enzyme leading to an allosteric activation of the enzyme after binding to methylated DNA. *J. Mol. Biol.*, **309**, 1189–1199.
51. Margot,J.B., Ehrenhofer-Murray,A.E. and Leonhardt,H. (2003) Interactions within the mammalian DNA methyltransferase family. *BMC Mol. Biol.*, **4**, 7.
52. Goyal,R., Reinhardt,R. and Jeltsch,A. (2006) Accuracy of DNA methylation pattern preservation by the Dnmt1 methyltransferase. *Nucleic Acids Res.*, **34**, 1182–1188.
53. Jeltsch,A. (2006) On the enzymatic properties of Dnmt1: specificity, processivity, mechanism of linear diffusion and allosteric regulation of the enzyme. *Epigenetics*, **1**, 63–66.
54. Bashtrykov,P., Rajavelu,A., Hackner,B., Ragozin,S., Carell,T. and Jeltsch,A. (2014) Targeted mutagenesis results in an activation of DNA methyltransferase 1 and confirms an autoinhibitory role of its RFTS domain. *Chembiochem*, **15**, 743–748.
55. Hermann,A., Goyal,R. and Jeltsch,A. (2004) The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. *J. Biol. Chem.*, **279**, 48350–48359.
56. Vilkaitis,G., Suetake,I., Klimasauskas,S. and Tajima,S. (2005) Processive methylation of hemimethylated CpG sites by mouse Dnmt1 DNA methyltransferase. *J. Biol. Chem.*, **280**, 64–72.
57. Liu,Y., Oakeley,E.J., Sun,L. and Jost,J.P. (1998) Multiple domains are involved in the targeting of the mouse DNA methyltransferase to the DNA replication foci. *Nucleic Acids Res.*, **26**, 1038–1045.
58. Iida,T., Suetake,I., Tajima,S., Morioka,H., Ohta,S., Obuse,C. and Tsurimoto,T. (2002) PCNA clamp facilitates action of DNA cytosine methyltransferase 1 on hemimethylated DNA. *Genes Cells*, **7**, 997–1007.
59. O'Keefe,R.T., Henderson,S.C. and Spector,D.L. (1992) Dynamic organization of DNA replication in mammalian cell nuclei: Spatially and temporally defined replication of chromosome-specific alpha-satellite DNA sequences. *J. Cell Biol.*, **116**, 1095–1110.
60. Bostick,M., Kim,J.K., Esteve,P.O., Clark,A., Pradhan,S. and Jacobsen,S.E. (2007) UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science*, **317**, 1760–1764.
61. Sharif,J., Muto,M., Takebayashi,S., Suetake,I., Iwamatsu,A., Endo,T.A., Shinga,J., Mizutani-Koseki,Y., Toyoda,T., Okamura,K. *et al.* (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature*, **450**, 908–912.
62. Avvakumov,G.V., Walker,J.R., Xue,S., Li,Y., Duan,S., Bronner,C., Arrowsmith,C.H. and Dhe-Paganon,S. (2008) Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. *Nature*, **455**, 822–825.
63. Hashimoto,H., Horton,J.R., Zhang,X., Bostick,M., Jacobsen,S.E. and Cheng,X. (2008) The SRA domain of UHRF1 flips 5-methylcytosine out of the DNA helix. *Nature*, **455**, 826–829.
64. Arita,K., Ariyoshi,M., Tochio,H., Nakamura,Y. and Shirakawa,M. (2008) Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. *Nature*, **455**, 818–821.
65. Jeltsch,A. (2008) Reading and writing DNA methylation. *Nat. Struct. Mol. Biol.*, **15**, 1003–1004.
66. Nady,N., Lemak,A., Walker,J.R., Avvakumov,G.V., Kareta,M.S., Achour,M., Xue,S., Duan,S., Allali-Hassani,A., Zuo,X. *et al.* (2011) Recognition of multivalent histone states associated with heterochromatin by UHRF1 protein. *J. Biol. Chem.*, **286**, 24300–24311.
67. Rothbart,S.B., Krajewski,K., Nady,N., Tempel,W., Xue,S., Badeaux,A.I., Barsyte-Lovejoy,D., Martinez,J.Y., Bedford,M.T., Fuchs,S.M. *et al.* (2012) Association of UHRF1 with methylated H3K9 directs the maintenance of DNA methylation. *Nat. Struct. Mol. Biol.*, **19**, 1155–1160.
68. Hu,L., Li,Z., Wang,P., Lin,Y. and Xu,Y. (2011) Crystal structure of PHD domain of UHRF1 and insights into recognition of unmodified histone H3 arginine residue 2. *Cell Res.*, **21**, 1374–1378.
69. Rajakumara,E., Wang,Z., Ma,H., Hu,L., Chen,H., Lin,Y., Guo,R., Wu,F., Li,H., Lan,F. *et al.* (2011) PHD finger recognition of unmodified histone H3R2 links UHRF1 to regulation of euchromatic gene expression. *Mol. Cell*, **43**, 275–284.
70. Wang,C., Shen,J., Yang,Z., Chen,P., Zhao,B., Hu,W., Lan,W., Tong,X., Wu,H., Li,G. *et al.* (2011) Structural basis for site-specific reading of unmodified R2 of histone H3 tail by UHRF1 PHD finger. *Cell Res.*, **21**, 1379–1382.
71. Qin,W., Wolf,P., Liu,N., Link,S., Smets,M., La Mastra,F., Forne,I., Pichler,G., Horl,D., Fellinger,K. *et al.* (2015) DNA methylation requires a DNMT1 ubiquitin interacting motif (UIM) and histone ubiquitination. *Cell Res.*, **25**, 911–929.
72. Rothbart,S.B., Dickson,B.M., Ong,M.S., Krajewski,K., Houliston,S., Kireev,D.B., Arrowsmith,C.H. and Strahl,B.D. (2013) Multivalent histone engagement by the linked tandem Tudor and PHD domains of UHRF1 is required for the epigenetic inheritance of DNA methylation. *Genes Dev.*, **27**, 1288–1298.
73. Liu,X., Gao,Q., Li,P., Zhao,Q., Zhang,J., Li,J., Koseki,H. and Wong,J. (2013) UHRF1 targets DNMT1 for DNA methylation through cooperative binding of hemi-methylated DNA and methylated H3K9. *Nat. Commun.*, **4**, 1563.
74. von Meyenn,F., Iurlaro,M., Habibi,E., Liu,N.Q., Salehzadeh-Yazdi,A., Santos,F., Petrini,E., Milagre,I., Yu,M., Xie,Z. *et al.* (2016) Impairment of DNA methylation maintenance is the main cause of global demethylation in naive embryonic stem cells. *Mol. Cell*, **62**, 983.
75. Nishiyama,A., Yamaguchi,L., Sharif,J., Johmura,Y., Kawamura,T., Nakanishi,K., Shimamura,S., Arita,K., Kodama,T., Ishikawa,F. *et al.* (2013) Uhrf1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication. *Nature*, **502**, 249–253.
76. Fang,J., Cheng,J.D., Wang,J.L., Zhang,Q., Liu,M.J., Gong,R., Wang,P., Zhang,X.D., Feng,Y.Y., Lan,W.X. *et al.* (2016) Hemi-methylated DNA opens a closed conformation of UHRF1 to facilitate its histone recognition. *Nat. Commun.*, **7**, 11197.
77. Du,Z., Song,J., Wang,Y., Zhao,Y., Guda,K., Yang,S., Kao,H.Y., Xu,Y., Willis,J., Markowitz,S.D. *et al.* (2010) DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. *Sci. Signal.*, **3**, ra80.
78. Felle,M., Joppien,S., Nemeth,A., Diermeier,S., Thalhammer,V., Dobner,T., Kremmer,E., Kappler,R. and Langst,G. (2011) The USP7/Dnmt1 complex stimulates the DNA methylation activity of Dnmt1 and regulates the stability of UHRF1. *Nucleic Acids Res.*, **39**, 8355–8365.
79. Qin,W., Leonhardt,H. and Spada,F. (2011) Usp7 and Uhrf1 control ubiquitination and stability of the maintenance DNA methyltransferase Dnmt1. *J. Cell. Biochem.*, **112**, 439–444.
80. Cheng,J., Yang,H., Fang,J., Ma,L., Gong,R., Wang,P., Li,Z. and Xu,Y. (2015) Molecular mechanism for USP7-mediated DNMT1 stabilization by acetylation. *Nat. Commun.*, **6**, 7023.
81. Nicholson,B. and Suresh Kumar,K.G. (2011) The multifaceted roles of USP7: new therapeutic opportunities. *Cell Biochem. Biophys.*, **60**, 61–68.
82. Fatemi,M., Hermann,A., Gowher,H. and Jeltsch,A. (2002) Dnmt3a and Dnmt1 functionally cooperate during de novo methylation of DNA. *Eur. J. Biochem.*, **269**, 4981–4984.
83. Christman,J.K., Sheikhnejad,G., Marasco,C.J. and Sufrin,J.R. (1995) 5-Methyl-2′-deoxycytidine in single-stranded DNA can act in cis to signal de novo DNA methylation. *Proc. Natl. Acad. Sc. U.S.A.*, **92**, 7347–7351.
84. Bacolla,A., Pradhan,S., Roberts,R.J. and Wells,R.D. (1999) Recombinant human DNA (cytosine-5) methyltransferase. II. Steady-state kinetics reveal allosteric activation by methylated dna. *J. Biol. Chem.*, **274**, 33011–33019.
85. Pradhan,S. and Esteve,P.O. (2003) Allosteric activator domain of maintenance human DNA (cytosine-5) methyltransferase and its role in methylation spreading. *Biochemistry*, **42**, 5321–5332.
86. Svedruzic,Z.M. and Reich,N.O. (2005) DNA cytosine C5 methyltransferase Dnmt1: Catalysis-dependent release of allosteric inhibition. *Biochemistry*, **44**, 9472–9485.
87. Flynn,J., Fang,J.Y., Mikovits,J.A. and Reich,N.O. (2003) A potent cell-active allosteric inhibitor of murine DNA cytosine C5 methyltransferase. *J. Biol. Chem.*, **278**, 8238–8243.
88. Bacolla,A., Pradhan,S., Larson,J.E., Roberts,R.J. and Wells,R.D. (2001) Recombinant human DNA (cytosine-5) methyltransferase. III. Allosteric control, reaction order, and influence of plasmid topology and triplet repeat length on methylation of the fragile X CGG.CCG sequence. *J. Biol. Chem.*, **276**, 18605–18613.
89. Eckhardt,F., Lewin,J., Cortese,R., Rakyan,V.K., Attwood,J., Burger,M., Burton,J., Cox,T.V., Davies,R., Down,T.A. *et al.* (2006)

DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat. Genet.*, **38**, 1378–1385.
90. Meissner,A., Mikkelsen,T.S., Gu,H., Wernig,M., Hanna,J., Sivachenko,A., Zhang,X., Bernstein,B.E., Nusbaum,C., Jaffe,D.B. *et al.* (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature*, **454**, 766–770.
91. Zhang,Y., Rohde,C., Tierling,S., Jurkowski,T.P., Bock,C., Santacruz,D., Ragozin,S., Reinhardt,R., Groth,M., Walter,J. *et al.* (2009) DNA methylation analysis of chromosome 21 gene promoters at single base pair and single allele resolution. *PLoS Genet.*, **5**, e1000438.
92. Glickman,J.F., Flynn,J. and Reich,N.O. (1997) Purification and characterization of recombinant baculovirus-expressed mouse DNA methyltransferase. *Biochem. Biophys. Res. Commun.*, **230**, 280–284.
93. Di Ruscio,A., Ebralidze,A.K., Benoukraf,T., Amabile,G., Goff,L.A., Terragni,J., Figueroa,M.E., De Figueiredo Pontes,L.L., Alberich-Jorda,M., Zhang,P. *et al.* (2013) DNMT1-interacting RNAs block gene-specific DNA methylation. *Nature*, **503**, 371–376.
94. Zhang,G., Esteve,P.O., Chin,H.G., Terragni,J., Dai,N., Correa,I.R. Jr and Pradhan,S. (2015) Small RNA-mediated DNA (cytosine-5) methyltransferase 1 inhibition leads to aberrant DNA methylation. *Nucleic Acids Res.*, **43**, 6112–6124.
95. Glickman,J.F., Pavlovich,J.G. and Reich,N.O. (1997) Peptide mapping of the murine DNA methyltransferase reveals a major phosphorylation site and the start of translation. *J. Biol. Chem.*, **272**, 17851–17857.
96. Goyal,R., Rathert,P., Laser,H., Gowher,H. and Jeltsch,A. (2007) Phosphorylation of serine-515 activates the Mammalian maintenance methyltransferase Dnmt1. *Epigenetics*, **2**, 155–160.
97. Sugiyama,Y., Hatano,N., Sueyoshi,N., Suetake,I., Tajima,S., Kinoshita,E., Kinoshita-Kikuta,E., Koike,T. and Kameshita,I. (2010) The DNA-binding activity of mouse DNA methyltransferase 1 is regulated by phosphorylation with casein kinase 1delta/epsilon. *Biochem. J.*, **427**, 489–497.
98. Hervouet,E., Lalier,L., Debien,E., Cheray,M., Geairon,A., Rogniaux,H., Loussouarn,D., Martin,S.A., Vallette,F.M. and Cartron,P.F. (2010) Disruption of Dnmt1/PCNA/UHRF1 interactions promotes tumorigenesis from human and mice glial cells. *PLoS One*, **5**, e11333.
99. Esteve,P.O., Chang,Y., Samaranayake,M., Upadhyay,A.K., Horton,J.R., Feehery,G.R., Cheng,X. and Pradhan,S. (2011) A methylation and phosphorylation switch between an adjacent lysine and serine determines human DNMT1 stability. *Nat. Struct. Mol. Biol.*, **18**, 42–48.
100. Esteve,P.O., Zhang,G., Ponnaluri,V.K., Deepti,K., Chin,H.G., Dai,N., Sagum,C., Black,K., Correa,I.R. Jr, Bedford,M.T. *et al.* (2016) Binding of 14-3-3 reader proteins to phosphorylated DNMT1 facilitates aberrant DNA methylation and gene expression. *Nucleic Acids Res.*, **44**, 1642–1656.
101. Lavoie,G., Esteve,P.O., Laulan,N.B., Pradhan,S. and St-Pierre,Y. (2011) PKC isoforms interact with and phosphorylate DNMT1. *BMC Biol.*, **9**, 31.
102. Kim,S.C., Sprung,R., Chen,Y., Xu,Y., Ball,H., Pei,J., Cheng,T., Kho,Y., Xiao,H., Xiao,L. *et al.* (2006) Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol. Cell*, **23**, 607–618.
103. Choudhary,C., Kumar,C., Gnad,F., Nielsen,M.L., Rehman,M., Walther,T.C., Olsen,J.V. and Mann,M. (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science*, **325**, 834–840.
104. Peng,L., Yuan,Z., Ling,H., Fukasawa,K., Robertson,K., Olashaw,N., Koomen,J., Chen,J., Lane,W.S. and Seto,E. (2011) SIRT1 deacetylates the DNA methyltransferase 1 (DNMT1) protein and alters its activities. *Mol. Cell. Biol.*, **31**, 4720–4734.
105. Zhou,Q., Agoston,A.T., Atadja,P., Nelson,W.G. and Davidson,N.E. (2008) Inhibition of histone deacetylases promotes ubiquitin-dependent proteasomal degradation of DNA methyltransferase 1 in human breast cancer cells. *Mol. Cancer Res.*, **6**, 873–883.
106. Esteve,P.O., Chin,H.G., Benner,J., Feehery,G.R., Samaranayake,M., Horwitz,G.A., Jacobsen,S.E. and Pradhan,S. (2009) Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.*, **106**, 5076–5081.
107. Wang,J., Hevi,S., Kurash,J.K., Lei,H., Gay,F., Bajko,J., Su,H., Sun,W., Chang,H., Xu,G. *et al.* (2009) The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat. Genet.*, **41**, 125–129.
108. Argentaro,A., Yang,J.C., Chapman,L., Kowalczyk,M.S., Gibbons,R.J., Higgs,D.R., Neuhaus,D. and Rhodes,D. (2007) Structural consequences of disease-causing mutations in the ATRX-DNMT3-DNMT3L (ADD) domain of the chromatin-associated protein ATRX. *Proc. Natl. Acad. Sci. U.S.A*, **104**, 11939–11944.
109. Hashimoto,H., Vertino,P.M. and Cheng,X. (2010) Molecular coupling of DNA methylation and histone methylation. *Epigenomics*, **2**, 657–669.
110. Ooi,S.K., Qiu,C., Bernstein,E., Li,K., Jia,D., Yang,Z., Erdjument-Bromage,H., Tempst,P., Lin,S.P., Allis,C.D. *et al.* (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature*, **448**, 714–717.
111. Otani,J., Nankumo,T., Arita,K., Inamoto,S., Ariyoshi,M. and Shirakawa,M. (2009) Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO Rep.*, **10**, 1235–1241.
112. Zhang,Y., Jurkowska,R., Soeroes,S., Rajavelu,A., Dhayalan,A., Bock,I., Rathert,P., Brandt,O., Reinhardt,R., Fischle,W. *et al.* (2010) Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic Acids Res.*, **38**, 4246–4253.
113. Guo,X., Wang,L., Li,J., Ding,Z., Xiao,J., Yin,X., He,S., Shi,P., Dong,L., Li,G. *et al.* (2015) Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature*, **517**, 640–644.
114. Dhayalan,A., Tamas,R., Bock,I., Tattermusch,A., Dimitrova,E., Kudithipudi,S., Ragozin,S. and Jeltsch,A. (2011) The ATRX-ADD domain binds to H3 tail peptides and reads the combined methylation state of K4 and K9. *Hum. Mol. Genet.*, **20**, 2195–2203.
115. Qin,S. and Min,J. (2014) Structure and function of the nucleosome-binding PWWP domain. *Trends Biochem. Sci.*, **39**, 536–547.
116. Qiu,C., Sawada,K., Zhang,X. and Cheng,X. (2002) The PWWP domain of mammalian DNA methyltransferase Dnmt3b defines a new family of DNA-binding folds. *Nat. Struct. Biol.*, **9**, 217–224.
117. Rondelet,G., Dal Maso,T., Willems,L. and Wouters,J. (2016) Structural basis for recognition of histone H3K36me3 nucleosome by human de novo DNA methyltransferases 3A and 3B. *J. Struct. Biol.*, **194**, 357–367.
118. Dhayalan,A., Rajavelu,A., Rathert,P., Tamas,R., Jurkowska,R.Z., Ragozin,S. and Jeltsch,A. (2010) The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *J. Biol. Chem.*, **285**, 26114–26120.
119. Baubec,T., Colombo,D.F., Wirbelauer,C., Schmidt,J., Burger,L., Krebs,A.R., Akalin,A. and Schubeler,D. (2015) Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature*, **520**, 243–247.
120. Suetake,I., Mishima,Y., Kimura,H., Lee,Y.H., Goto,Y., Takeshima,H., Ikegami,T. and Tajima,S. (2011) Characterization of DNA-binding activity in the N-terminal domain of the DNA methyltransferase Dnmt3a. *Biochem. J.*, **437**, 141–148.
121. Gowher,H. and Jeltsch,A. (2002) Molecular enzymology of the catalytic domains of the Dnmt3a and Dnmt3b DNA methyltransferases. *J. Biol. Chem.*, **277**, 20409–20414.
122. Li,B.Z., Huang,Z., Cui,Q.Y., Song,X.H., Du,L., Jeltsch,A., Chen,P., Li,G., Li,E. and Xu,G.L. (2011) Histone tails regulate DNA methylation by allosterically activating de novo methyltransferase. *Cell Res.*, **21**, 1172–1181.
123. Jia,D., Jurkowska,R.Z., Zhang,X., Jeltsch,A. and Cheng,X. (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature*, **449**, 248–251.
124. Jurkowska,R.Z., Anspach,N., Urbanke,C., Jia,D., Reinhardt,R., Nellen,W., Cheng,X. and Jeltsch,A. (2008) Formation of nucleoprotein filaments by mammalian DNA methyltransferase Dnmt3a in complex with regulator Dnmt3L. *Nucleic Acids Res.*, **36**, 6656–6663.

125. Cokus,S.J., Feng,S., Zhang,X., Chen,Z., Merriman,B., Haudenschild,C.D., Pradhan,S., Nelson,S.F., Pellegrini,M. and Jacobsen,S.E. (2008) Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. *Nature*, **452**, 215–219.
126. Lister,R., Pelizzola,M., Dowen,R.H., Hawkins,R.D., Hon,G., Tonti-Filippini,J., Nery,J.R., Lee,L., Ye,Z., Ngo,Q.M. *et al.* (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*, **462**, 315–322.
127. Chodavarapu,R.K., Feng,S., Bernatavichute,Y.V., Chen,P.Y., Stroud,H., Yu,Y., Hetzel,J.A., Kuo,F., Kim,J., Cokus,S.J. *et al.* (2010) Relationship between nucleosome positioning and DNA methylation. *Nature*, **466**, 388–392.
128. Smallwood,S.A., Tomizawa,S., Krueger,F., Ruf,N., Carli,N., Segonds-Pichon,A., Sato,S., Hata,K., Andrews,S.R. and Kelsey,G. (2011) Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat. Genet.*, **43**, 811–814.
129. Aoki,A., Suetake,I., Miyagawa,J., Fujio,T., Chijiwa,T., Sasaki,H. and Tajima,S. (2001) Enzymatic properties of de novo-type mouse DNA (cytosine-5) methyltransferases. *Nucleic Acids Res.*, **29**, 3506–3512.
130. Ramsahoye,B.H., Biniszkiewicz,D., Lyko,F., Clark,V., Bird,A.P. and Jaenisch,R. (2000) Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 5237–5242.
131. Varley,K.E., Gertz,J., Bowling,K.M., Parker,S.L., Reddy,T.E., Pauli-Behn,F., Cross,M.K., Williams,B.A., Stamatoyannopoulos,J.A., Crawford,G.E. *et al.* (2013) Dynamic DNA methylation across diverse human cell lines and tissues. *Genome Res.*, **23**, 555–567.
132. Lister,R., Mukamel,E.A., Nery,J.R., Urich,M., Puddifoot,C.A., Johnson,N.D., Lucero,J., Huang,Y., Dwork,A.J., Schultz,M.D. *et al.* (2013) Global epigenomic reconfiguration during mammalian brain development. *Science*, **341**, 1237905.
133. Guo,J.U., Su,Y., Shin,J.H., Shin,J., Li,H., Xie,B., Zhong,C., Hu,S., Le,T., Fan,G. *et al.* (2014) Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. *Nat. Neurosci.*, **17**, 215–222.
134. Schultz,M.D., He,Y., Whitaker,J.W., Hariharan,M., Mukamel,E.A., Leung,D., Rajagopal,N., Nery,J.R., Urich,M.A., Chen,H. *et al.* (2015) Human body epigenome maps reveal noncanonical DNA methylation variation. *Nature*, **523**, 212–216.
135. Ziller,M.J., Muller,F., Liao,J., Zhang,Y., Gu,H., Bock,C., Boyle,P., Epstein,C.B., Bernstein,B.E., Lengauer,T. *et al.* (2011) Genomic distribution and inter-sample variation of non-CpG methylation across human cell types. *PLoS Genet.*, **7**, e1002389.
136. Arand,J., Spieler,D., Karius,T., Branco,M.R., Meilinger,D., Meissner,A., Jenuwein,T., Xu,G., Leonhardt,H., Wolf,V. *et al.* (2012) In vivo control of CpG and non-CpG DNA methylation by DNA methyltransferases. *PLoS Genet.*, **8**, e1002750.
137. Gabel,H.W., Kinde,B., Stroud,H., Gilbert,C.S., Harmin,D.A., Kastan,N.R., Hemberg,M., Ebert,D.H. and Greenberg,M.E. (2015) Disruption of DNA-methylation-dependent long gene repression in Rett syndrome. *Nature*, **522**, 89–93.
138. Chen,L., Chen,K., Lavery,L.A., Baker,S.A., Shaw,C.A., Li,W. and Zoghbi,H.Y. (2015) MeCP2 binds to non-CG methylated DNA as neurons mature, influencing transcription and the timing of onset for Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.*, **112**, 5509–5514.
139. Jurkowska,R.Z., Siddique,A.N., Jurkowski,T.P. and Jeltsch,A. (2011) Approaches to enzyme and substrate design of the murine Dnmt3a DNA methyltransferase. *Chembiochem*, **12**, 1589–1594.
140. Lin,I.G., Han,L., Taghva,A., O'Brien,L.E. and Hsieh,C.L. (2002) Murine de novo methyltransferase Dnmt3a demonstrates strand asymmetry and site preference in the methylation of DNA in vitro. *Mol. Cell. Biol.*, **22**, 704–723.
141. Handa,V. and Jeltsch,A. (2005) Profound flanking sequence preference of Dnmt3a and Dnmt3b mammalian DNA methyltransferases shape the human epigenome. *J. Mol. Biol.*, **348**, 1103–1112.
142. Holz-Schietinger,C. and Reich,N.O. (2010) The inherent processivity of the human de novo methyltransferase 3A (DNMT3A) is enhanced by DNMT3L. *J. Biol. Chem.*, **285**, 29091–29100.
143. Rajavelu,A., Jurkowska,R.Z., Fritz,J. and Jeltsch,A. (2012) Function and disruption of DNA methyltransferase 3a cooperative DNA binding and nucleoprotein filament formation. *Nucleic Acids Res.*, **40**, 569–580.
144. Emperle,M., Rajavelu,A., Reinhardt,R., Jurkowska,R.Z. and Jeltsch,A. (2014) Cooperative DNA binding and protein/DNA fiber formation increases the activity of the Dnmt3a DNA methyltransferase. *J. Biol. Chem.*, **289**, 29602–29613.
145. Jeltsch,A. and Jurkowska,R.Z. (2013) Multimerization of the dnmt3a DNA methyltransferase and its functional implications. *Prog. Mol. Biol. Transl. Sci.*, **117**, 445–464.
146. Gowher,H., Stockdale,C.J., Goyal,R., Ferreira,H., Owen-Hughes,T. and Jeltsch,A. (2005) De novo methylation of nucleosomal DNA by the mammalian Dnmt1 and Dnmt3A DNA methyltransferases. *Biochemistry*, **44**, 9899–9904.
147. Takeshima,H., Suetake,I. and Tajima,S. (2008) Mouse Dnmt3a preferentially methylates linker DNA and is inhibited by histone H1. *J. Mol. Biol.*, **383**, 810–821.
148. Felle,M., Hoffmeister,H., Rothammer,J., Fuchs,A., Exler,J.H. and Langst,G. (2011) Nucleosomes protect DNA from DNA methylation in vivo and in vitro. *Nucleic Acids Res.*, **39**, 6956–6969.
149. Morselli,M., Pastor,W.A., Montanini,B., Nee,K., Ferrari,R., Fu,K., Bonora,G., Rubbi,L., Clark,A.T., Ottonello,S. *et al.* (2015) In vivo targeting of de novo DNA methylation by histone modifications in yeast and mouse. *Elife*, **4**, e06205.
150. Datta,J., Majumder,S., Bai,S., Ghoshal,K., Kutay,H., Smith,D.S., Crabb,J.W. and Jacob,S.T. (2005) Physical and functional interaction of DNA methyltransferase 3A with Mbd3 and Brg1 in mouse lymphosarcoma cells. *Cancer Res.*, **65**, 10891–10900.
151. Cai,Y., Geutjes,E.J., de Lint,K., Roepman,P., Bruurs,L., Yu,L.R., Wang,W., van Blijswijk,J., Mohammad,H., de Rink,I. *et al.* (2014) The NuRD complex cooperates with DNMTs to maintain silencing of key colorectal tumor suppressor genes. *Oncogene*, **33**, 2157–2168.
152. Geiman,T.M., Sankpal,U.T., Robertson,A.K., Zhao,Y. and Robertson,K.D. (2004) DNMT3B interacts with hSNF2H chromatin remodeling enzyme, HDACs 1 and 2, and components of the histone methylation system. *Biochem. Biophys. Res. Commun.*, **318**, 544–555.
153. Zhu,H., Geiman,T.M., Xi,S., Jiang,Q., Schmidtmann,A., Chen,T., Li,E. and Muegge,K. (2006) Lsh is involved in de novo methylation of DNA. *EMBO J.*, **25**, 335–345.
154. Myant,K. and Stancheva,I. (2008) LSH cooperates with DNA methyltransferases to repress transcription. *Mol. Cell. Biol.*, **28**, 215–226.
155. Muegge,K. (2005) Lsh, a guardian of heterochromatin at repeat elements. *Biochem. Cell Biol.*, **83**, 548–554.
156. Termanis,A., Torrea,N., Culley,J., Kerr,A., Ramsahoye,B. and Stancheva,I. (2016) The SNF2 family ATPase LSH promotes cell-autonomous de novo DNA methylation in somatic cells. *Nucleic Acids Res.*, doi:10.1093/nar/gkw424.
157. Jurkowska,R.Z., Rajavelu,A., Anspach,N., Urbanke,C., Jankevicius,G., Ragozin,S., Nellen,W. and Jeltsch,A. (2011) Oligomerization and binding of the Dnmt3a DNA methyltransferase to parallel DNA molecules: heterochromatic localization and role of Dnmt3L. *J. Biol. Chem.*, **286**, 24200–24207.
158. Kareta,M.S., Botello,Z.M., Ennis,J.J., Chou,C. and Chedin,F. (2006) Reconstitution and mechanism of the stimulation of de novo methylation by human DNMT3L. *J. Biol. Chem.*, **281**, 25893–25902.
159. Li,J.Y., Pu,M.T., Hirasawa,R., Li,B.Z., Huang,Y.N., Zeng,R., Jing,N.H., Chen,T., Li,E., Sasaki,H. *et al.* (2007) Synergistic function of DNA methyltransferases Dnmt3a and Dnmt3b in the methylation of Oct4 and Nanog. *Mol. Cell. Biol.*, **27**, 8748–8759.
160. Noh,K.M., Wang,H., Kim,H.R., Wenderski,W., Fang,F., Li,C.H., Dewell,S., Hughes,S.H., Melnick,A.M., Patel,D.J. *et al.* (2015) Engineering of a histone-recognition domain in Dnmt3a alters the epigenetic landscape and phenotypic features of mouse ESCs. *Mol. Cell*, **59**, 89–103.
161. Hodges,E., Smith,A.D., Kendall,J., Xuan,Z., Ravi,K., Rooks,M., Zhang,M.Q., Ye,K., Bhattacharjee,A., Brizuela,L. *et al.* (2009) High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. *Genome Res.*, **19**, 1593–1605.

162. Weber,M., Hellmann,I., Stadler,M.B., Ramos,L., Paabo,S., Rebhan,M. and Schubeler,D. (2007) Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.*, **39**, 457–466.
163. Barski,A., Cuddapah,S., Cui,K., Roh,T.Y., Schones,D.E., Wang,Z., Wei,G., Chepelev,I. and Zhao,K. (2007) High-resolution profiling of histone methylations in the human genome. *Cell*, **129**, 823–837.
164. Edmunds,J.W., Mahadevan,L.C. and Clayton,A.L. (2008) Dynamic histone H3 methylation during gene induction: HYPB/Setd2 mediates all H3K36 trimethylation. *EMBO J.*, **27**, 406–420.
165. Guenther,M.G., Levine,S.S., Boyer,L.A., Jaenisch,R. and Young,R.A. (2007) A chromatin landmark and transcription initiation at most promoters in human cells. *Cell*, **130**, 77–88.
166. Larschan,E., Alekseyenko,A.A., Gortchakov,A.A., Peng,S., Li,B., Yang,P., Workman,J.L., Park,P.J. and Kuroda,M.I. (2007) MSL complex is attracted to genes marked by H3K36 trimethylation using a sequence-independent mechanism. *Mol. Cell*, **28**, 121–133.
167. Vakoc,C.R., Sachdeva,M.M., Wang,H. and Blobel,G.A. (2006) Profile of histone lysine methylation across transcribed mammalian chromatin. *Mol. Cell. Biol.*, **26**, 9185–9195.
168. Ball,M.P., Li,J.B., Gao,Y., Lee,J.H., LeProust,E.M., Park,I.H., Xie,B., Daley,G.Q. and Church,G.M. (2009) Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat. Biotechnol.*, **27**, 361–368.
169. Hellman,A. and Chess,A. (2007) Gene body-specific methylation on the active X chromosome. *Science*, **315**, 1141–1143.
170. Kolasinska-Zwierz,P., Down,T., Latorre,I., Liu,T., Liu,X.S. and Ahringer,J. (2009) Differential chromatin marking of introns and expressed exons by H3K36me3. *Nat. Genet.*, **41**, 376–381.
171. Ernst,J., Kheradpour,P., Mikkelsen,T.S., Shoresh,N., Ward,L.D., Epstein,C.B., Zhang,X., Wang,L., Issner,R., Coyne,M. *et al.* (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature*, **473**, 43–49.
172. Purdy,M.M., Holz-Schietinger,C. and Reich,N.O. (2010) Identification of a second DNA binding site in human DNA methyltransferase 3A by substrate inhibition and domain deletion. *Arch. Biochem. Biophys.*, **498**, 13–22.
173. van Nuland,R., van Schaik,F.M., Simonis,M., van Heesch,S., Cuppen,E., Boelens,R., Timmers,H.M. and van Ingen,H. (2013) Nucleosomal DNA binding drives the recognition of H3K36-methylated nucleosomes by the PSIP1-PWWP domain. *Epigenetics Chromatin*, **6**, 12.
174. Fuks,F., Hurd,P.J., Deplus,R. and Kouzarides,T. (2003) The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res.*, **31**, 2305–2312.
175. Li,H., Rauch,T., Chen,Z.X., Szabo,P.E., Riggs,A.D. and Pfeifer,G.P. (2006) The histone methyltransferase SETDB1 and the DNA methyltransferase DNMT3A interact directly and localize to promoters silenced in cancer cells. *J. Biol. Chem.*, **281**, 19489–19500.
176. Vire,E., Brenner,C., Deplus,R., Blanchon,L., Fraga,M., Didelot,C., Morey,L., Van Eynde,A., Bernard,D., Vanderwinden,J.M. *et al.* (2006) The Polycomb group protein EZH2 directly controls DNA methylation. *Nature*, **439**, 871–874.
177. Kimura,H. and Shiota,K. (2003) Methyl-CpG-binding protein, MeCP2, is a target molecule for maintenance DNA methyltransferase, Dnmt1. *J. Biol. Chem.*, **278**, 4806–4812.
178. Fuks,F., Hurd,P.J., Wolf,D., Nan,X., Bird,A.P. and Kouzarides,T. (2003) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J. Biol. Chem.*, **278**, 4035–4040.
179. Suzuki,M., Yamada,T., Kihara-Negishi,F., Sakurai,T., Hara,E., Tenen,D.G., Hozumi,N. and Oikawa,T. (2006) Site-specific DNA methylation by a complex of PU.1 and Dnmt3a/b. *Oncogene*, **25**, 2477–2488.
180. Brenner,C., Deplus,R., Didelot,C., Loriot,A., Vire,E., De Smet,C., Gutierrez,A., Danovi,D., Bernard,D., Boon,T. *et al.* (2005) Myc represses transcription through recruitment of DNA methyltransferase corepressor. *EMBO J.*, **24**, 336–346.
181. Fuks,F., Burgers,W.A., Godin,N., Kasai,M. and Kouzarides,T. (2001) Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription. *EMBO J.*, **20**, 2536–2544.
182. Kim,S.H., Park,J., Choi,M.C., Kim,H.P., Park,J.H., Jung,Y., Lee,J.H., Oh,D.Y., Im,S.A., Bang,Y.J. *et al.* (2007) Zinc-fingers and homeoboxes 1 (ZHX1) binds DNA methyltransferase (DNMT) 3B to enhance DNMT3B-mediated transcriptional repression. *Biochem. Biophys. Res. Commun.*, **355**, 318–323.
183. Lehnertz,B., Ueda,Y., Derijck,A.A., Braunschweig,U., Perez-Burgos,L., Kubicek,S., Chen,T., Li,E., Jenuwein,T. and Peters,A.H. (2003) Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr. Biol.*, **13**, 1192–1200.
184. Hata,K., Okano,M., Lei,H. and Li,E. (2002) Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development*, **129**, 1983–1993.
185. Chedin,F., Lieber,M.R. and Hsieh,C.L. (2002) The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 16916–16921.
186. Chen,Z.X., Mann,J.R., Hsieh,C.L., Riggs,A.D. and Chedin,F. (2005) Physical and functional interactions between the human DNMT3L protein and members of the de novo methyltransferase family. *J. Cell. Biochem.*, **95**, 902–917.
187. Suetake,I., Shinozaki,F., Miyagawa,J., Takeshima,H. and Tajima,S. (2004) DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. *J. Biol. Chem.*, **279**, 27816–27823.
188. Gowher,H., Liebert,K., Hermann,A., Xu,G. and Jeltsch,A. (2005) Mechanism of stimulation of catalytic activity of Dnmt3A and Dnmt3B DNA-(cytosine-C5)-methyltransferases by Dnmt3L. *J. Biol. Chem.*, **280**, 13341–13348.
189. Bourc'his,D., Xu,G.L., Lin,C.S., Bollman,B. and Bestor,T.H. (2001) Dnmt3L and the establishment of maternal genomic imprints. *Science*, **294**, 2536–2539.
190. Bourc'his,D. and Bestor,T.H. (2004) Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature*, **431**, 96–99.
191. Neri,F., Krepelova,A., Incarnato,D., Maldotti,M., Parlato,C., Galvagni,F., Matarese,F., Stunnenberg,H.G. and Oliviero,S. (2013) Dnmt3L antagonizes DNA methylation at bivalent promoters and favors DNA methylation at gene bodies in ESCs. *Cell*, **155**, 121–134.
192. Duymich,C.E., Charlet,J., Yang,X.J., Jones,P.A. and Liang,G.N. (2016) DNMT3B isoforms without catalytic activity stimulate gene body methylation as accessory proteins in somatic cells. *Nat. Commun.*, **7**, 11453.
193. Holz-Schietinger,C. and Reich,N.O. (2012) RNA modulation of the human DNA methyltransferase 3A. *Nucleic Acids Res.*, **40**, 8550–8557.
194. Schmitz,K.M., Mayer,C., Postepska,A. and Grummt,I. (2010) Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes. *Genes Dev.*, **24**, 2264–2269.
195. Bierhoff,H., Schmitz,K., Maass,F., Ye,J. and Grummt,I. (2010) Noncoding transcripts in sense and antisense orientation regulate the epigenetic state of ribosomal RNA genes. *Cold Spring Harb. Symp. Quant. Biol.*, **75**, 357–364.
196. Deplus,R., Blanchon,L., Rajavelu,A., Boukaba,A., Defrance,M., Luciani,J., Rothe,F., Dedeurwaerder,S., Denis,H., Brinkman,A.B. *et al.* (2014) Regulation of DNA methylation patterns by CK2-mediated phosphorylation of Dnmt3a. *Cell Rep.*, **8**, 743–753.
197. Li,Y., Han,J., Zhang,Y., Cao,F., Liu,Z., Li,S., Wu,J., Hu,C., Wang,Y., Shuai,J. *et al.* (2016) Structural basis for activity regulation of MLL family methyltransferases. *Nature*, **530**, 447–452.
198. Muller,M.M., Fierz,B., Bittova,L., Liszczak,G. and Muir,T.W. (2016) A two-state activation mechanism controls the histone methyltransferase Suv39h1. *Nat. Chem. Biol.*, **12**, 188–193.
199. Jiao,L. and Liu,X. (2015) Structural basis of histone H3K27 trimethylation by an active polycomb repressive complex 2. *Science*, **350**, aac4383.
200. Holoch,D. and Moazed,D. (2015) RNA-mediated epigenetic regulation of gene expression. *Nat. Rev. Genet.*, **16**, 71–84.
201. Rinn,J.L. and Chang,H.Y. (2012) Genome regulation by long noncoding RNAs. *Annu Rev Biochem*, **81**, 145–166.
202. Matzke,M.A. and Mosher,R.A. (2014) RNA-directed DNA methylation: An epigenetic pathway of increasing complexity. *Nat. Rev. Genet.*, **15**, 394–408.
203. Iwasaki,Y.W., Siomi,M.C. and Siomi,H. (2015) PIWI-Interacting RNA: Its biogenesis and functions. *Annu. Rev. Biochem.*, **84**, 405–433.