



Domain Structure of the Dnmt1, Dnmt3a, and Dnmt3b DNA Methyltransferases

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Abstract

In mammals, three major DNA methyltransferases, Dnmt1, Dnmt3a, and Dnmt3b, have been identified. Dnmt3a and Dnmt3b are responsible for establishing DNA methylation patterns produced through their de novo-type DNA methylation activity in implantation stage embryos and during germ cell differentiation. Dnmt3-like (Dnmt3l), which is a member of the Dnmt3 family but does not possess DNA methylation activity, was reported to be indispensable for global methylation in germ cells. Once the DNA methylation patterns are established, maintenance-type DNA methyltransferase Dnmt1 faithfully propagates them to the next generation via replication. All Dnmts possess

multiple domains. For instance, Dnmt3a and Dnmt3b each contain a Pro-Trp-Trp-Pro (PWWP) domain that recognizes the histone H3K36me2/3 mark, an Atrp-Dnmt3-Dnmt3l (ADD) domain that recognizes unmodified histone H3 tail, and a catalytic domain that methylates CpG sites. Dnmt1 contains an N-terminal independently folded domain (NTD) that interacts with a variety of regulatory factors, a replication foci-targeting sequence (RFTS) domain that recognizes the histone H3K9me3 mark and H3 ubiquitylation, a CXXC domain that recognizes unmodified CpG DNA, two tandem Bromo-Adjacent-homology (BAH1 and BAH2) domains that read the H4K20me3 mark with BAH1, and a catalytic domain that preferentially methylates hemimethylated CpG sites. In this chapter, the structures and functions of these domains are described.

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recognition domain · Target cytosine

Abbreviations

AdoMet	S-Adenosyl-L-methionine
BAH	Bromo-adjacent-homology domain
domain	

DMR	Differentially methylated region
ES cells	Embryonic stem cells
ICF syndrome	Immunodeficiency, centromeric instability, and facial anomalies syndrome
NTD	The N-terminal independently folded domain
RFTS domain	Replication foci-targeting sequence domain
SAH	S-Adenosyl-L-homocysteine
SRA domain	The SET and RING-associated domain
TDG	Thymine DNA glycosylase
Tet enzyme	Ten-eleven translocation enzyme
TRD	Target recognition domain

3.1 DNA Methylation and Methyltransferases in Mammals

The methylation patterns of genomic DNA are established at an early stage of embryogenesis. Once the global methylation patterns are established, they are maintained during replication in a cell lineage-dependent manner (Fig. 3.1a). In mammals, a second methylation reprogramming occurs in gametogenesis. The global DNA methylation patterns are removed during an early stage of germ cell development and reestablished before meiosis in gonocytes in males and growing oocytes in females (Bird 2002). The expression of more than a hundred genes on autosomes is regulated in a sex-dependent manner, these genes being called imprinted genes. These genes are characterized by differentially methylated regions (DMRs), which undergo distinct DNA methylation in the male and female genomes. Generally, the DMR methylation patterns are established in germ cells at an identical stage to that of global DNA methylation (Kaneda et al. 2004). In mammals, three major DNA methyltransferases, Dnmt1, Dnmt3a, and Dnmt3b, have been identified (Bestor et al. 1988; Okano et al. 1998). Dnmt3a and Dnmt3b are responsible for establishing DNA methylation

patterns produced through their de novo-type DNA methylation activity in implantation stage embryos and during germ cell differentiation (Okano et al. 1999). In addition, Dnmt3c, an enzyme closely related to Dnmt3b, methylates evolutionarily young transposons in the mouse male germ line (Barau et al. 2016; Jain et al. 2017). Dnmt3-like (Dnmt3l), which is a member of the Dnmt3 family but does not possess DNA methylation activity, was reported to be indispensable for global methylation in germ cells (Bourc'his et al. 2001; Hata et al. 2002). Once the DNA methylation patterns are established, the maintenance-type DNA methyltransferase Dnmt1 faithfully propagates them to the next generation after DNA replication. Dnmt1 preferentially methylates hemimethylated CpG sites, which appear after DNA replication and repair.

3.2 Enzymes Responsible for the Establishment of DNA Methylation Patterns

In mammals, the establishment of DNA methylation patterns is mainly mediated by the de novo DNA-(cytosine C5)-methyltransferases Dnmt3a and Dnmt3b, which are encoded in distinct gene loci (Aoki et al. 2001; Okano et al. 1999). Their domain arrangements are similar, each comprising a Pro-Trp-Trp-Pro (PWWP), Atrx-Dnmt3-Dnmt3l (ADD), and C-terminal catalytic domain (Fig. 3.1b). The PWWP domain is reported to bind to DNA (Qiu et al. 2002) and histone tails (Dhayalan et al. 2010) and the ADD domain to interact with various proteins including histone tails, as described below (Brenner et al. 2005; Fuks et al. 2001; Otani et al. 2009). In addition, Dnmt3a isoform 1 (Dnmt3a1) contains a ubiquitin-dependent recruitment region (UDR) that binds to monoubiquitylated histone H2A lysine 119 (H2AK119ub) (Fig. 3.1b) (Weinberg et al. 2021). Dnmt3l, a homolog of Dnmt3a and Dnmt3b, possesses no conserved domain for DNA methylation but contains an ADD domain (Aapola et al. 2000) and is necessary for global

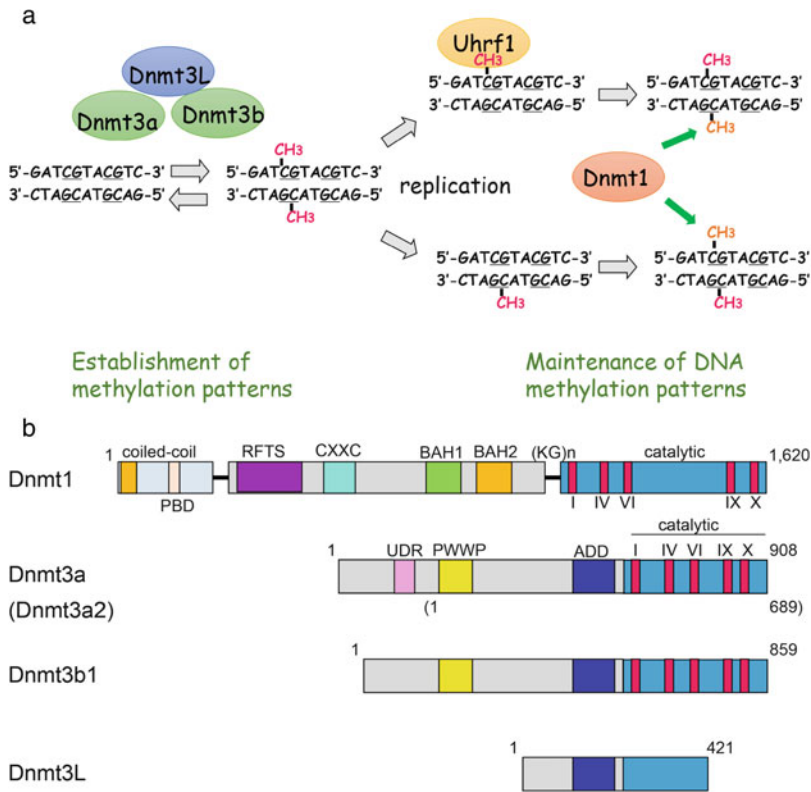


Fig. 3.1 Schematic illustration of establishment and maintenance of DNA methylation patterns. (a) The methylation patterns of genomic DNA are established at an early stage of embryogenesis by de novo-type DNA methyltransferases, Dnmt3a and Dnmt3b, with the aid of Dnmt3L. Once the global methylation patterns are established, they are maintained during replication by maintenance DNA methyltransferase Dnmt1 in

collaboration with Uhrf1 in a cell lineage-dependent manner. (b) Schematic illustration of mammalian DNA methyltransferases, Dnmt1, Dnmt3a, and Dnmt3b. Dnmt3a has a short isoform utilizing different promoter and a transcription start site, Dnmt3a2. Dnmt3L, a member of the Dnmt3 family, lacks the catalytic domain and thus does not exhibit DNA methylation activity

DNA methylation (Bourc'his et al. 2001; Hata et al. 2002).

3.2.1 PWWP Domain

The PWWP domain of Dnmt3 enzymes, comprising 100–150 amino acid residues, is characterized by a central core sequence motif of Pro-Trp-Trp-Pro. It was hypothesized that the domain contributes to protein–protein interactions, especially of proteins involved in cell division, growth, and differentiation, based on a comparison of 39 proteins containing a PWWP domain (Stec et al. 2000). The PWWP domains of

Dnmt3a and Dnmt3b tether them to chromatin regions (Ge et al. 2004), especially to pericentric heterochromatin and thus are responsible for their DNA methylation (Chen et al. 2004).

The PWWP domain of Dnmt3b, comprising a beta-barrel structure with 5 beta-strands followed by a five-helix bundle, turned out to be a fold responsible for DNA binding (Qiu et al. 2002). Positively charged Lys and Arg residues on the surface of the domain are expected to be the sites for DNA binding. The beta-barrel part of the PWWP domain is homologous to that of the SAND domain (named after Sp100, AIRE-1, NucP41/75, DEAF-1), which is a DNA-binding motif, and the Tudor domain, which is generally a

histone-binding motif. The PWWP domain of Dnmt3b binds to histone H3 tri-methylated at lysine 36 (H3K36me3) via a hydrophobic cage (Fig. 3.2a) (Rondelet et al. 2016), which is responsible for the recruitment of Dnmt3b, but not that of Dnmt3a, to the H3K36me3-containing gene body for de novo methylation (Baubec et al. 2015). A point mutation in the PWWP domain in Dnmt3b was found to be the cause of the immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome (Shirohzu et al. 2002), which is the consequence of hypomethylation of the pericentromere (Hansen et al. 1999; Okano et al. 1999).

The PWWP domain of Dnmt3a is highly homologous to that of Dnmt3b, possessing a corresponding hydrophobic cage (Fig. 3.2b). The Dnmt3a PWWP domain binds to both H3K36me2 and H3K36me3, with a subtle preference toward H3K36me2 (Dhayalan et al. 2010; Dukatz et al. 2019; Weinberg et al. 2019). The PWWP-H3K36me2 interaction ensures DNA methylation at the intergenic repeats (Weinberg et al. 2019). The PWWP domain of Dnmt3a also binds to DNA, which is important for chromatin association of Dnmt3a, though the affinity toward DNA is one order of magnitude lower compared to that of the PWWP domain of Dnmt3b (Dukatz et al. 2019; Purdy et al. 2010).

ZHX1, a member of the zinc finger and homeobox protein family, interacts with the PWWP domain of Dnmt3b and contributes to gene silencing (Kim et al. 2007). In addition, thymine DNA glycosylase (TDG), which is a T/G mismatch glycosylase, interacts with the PWWP and/or catalytic domains of Dnmt3a to modulate its DNA methylation activity. TDG was postulated to be responsible for the removal of formylcytosine and carboxylcytosine, which are the oxidation products of methylcytosine via hydroxymethylcytosine for active demethylation initiated by Ten-eleven translocation (Tet) enzymes (He et al. 2011; Maiti and Drohat 2011). The interaction between TDG and Dnmt3a suggests their functional interplay.

3.2.2 ADD Domain

The plant homeodomain (PHD)-like ADD domain is rich in Cys residues and reportedly binds to many factors. The ADD domain of Dnmt3a was reported to bind to corepressor RP58 (Fuks et al. 2001), oncogene c-myc (Brenner et al. 2005), Lys 9 histone H3 (H3K9) methylase Suv39h1 and heterochromatin protein 1 (HP1) beta (Fuks et al. 2003), H3K9 methylase Setdb1 (Li et al. 2006), or histone H3 un-methylated at Lys 4 (H3K4me0) (Otani et al. 2009; Zhang et al. 2010).

The three-dimensional structure of the ADD domain of Dnmt3a is similar to those of Dnmt3l and ATRX (Argentaro et al. 2007; Ooi et al. 2007) (Fig. 3.2c) and possibly Dnmt3b as well (Zhang et al. 2010). The affinity of the ADD domain of Dnmt3a to histone H3 tail is in the sub-micromolar range and is decreased by methylation modification at Lys 4 (Otani et al. 2009). This explains why the H3K4me3, which is a mark associated with active gene promoters, protects DNA from methylation (Okitsu and Hsieh 2007; Weber et al. 2007). X-ray crystallography showed that the histone H3 tail fits into the shallow groove of the PHD finger motif in the ADD domain. The main chain of Arg 2 to Thr 6 of histone H3 forms hydrogen bonds with the ADD, and this induces a conformational change of the ADD (Otani et al. 2009). The mode of recognition of the H3K4me0 by the ADD domain of Dnmt3a is similar to that of Dnmt3l (Fig. 3.2d), although the affinity is tenfold higher. As described below, Dnmt3l interacts directly with Dnmt3a and Dnmt3b (Suetake et al. 2004), and the proteins exist as a complex in embryonic stem (ES) cells (Li et al. 2007). Selective recognition of H3K4me0 by the ADD domains of Dnmt3a (Dnmt3b) and Dnmt3l may recruit de novo methyltransferases to the sites to be methylated. Conversion of the Dnmt3a ADD domain into a H3 Lys 4 methylation or H3 Thr 3 phosphorylation-insensitive module via protein engineering led to altered gene expression and/or

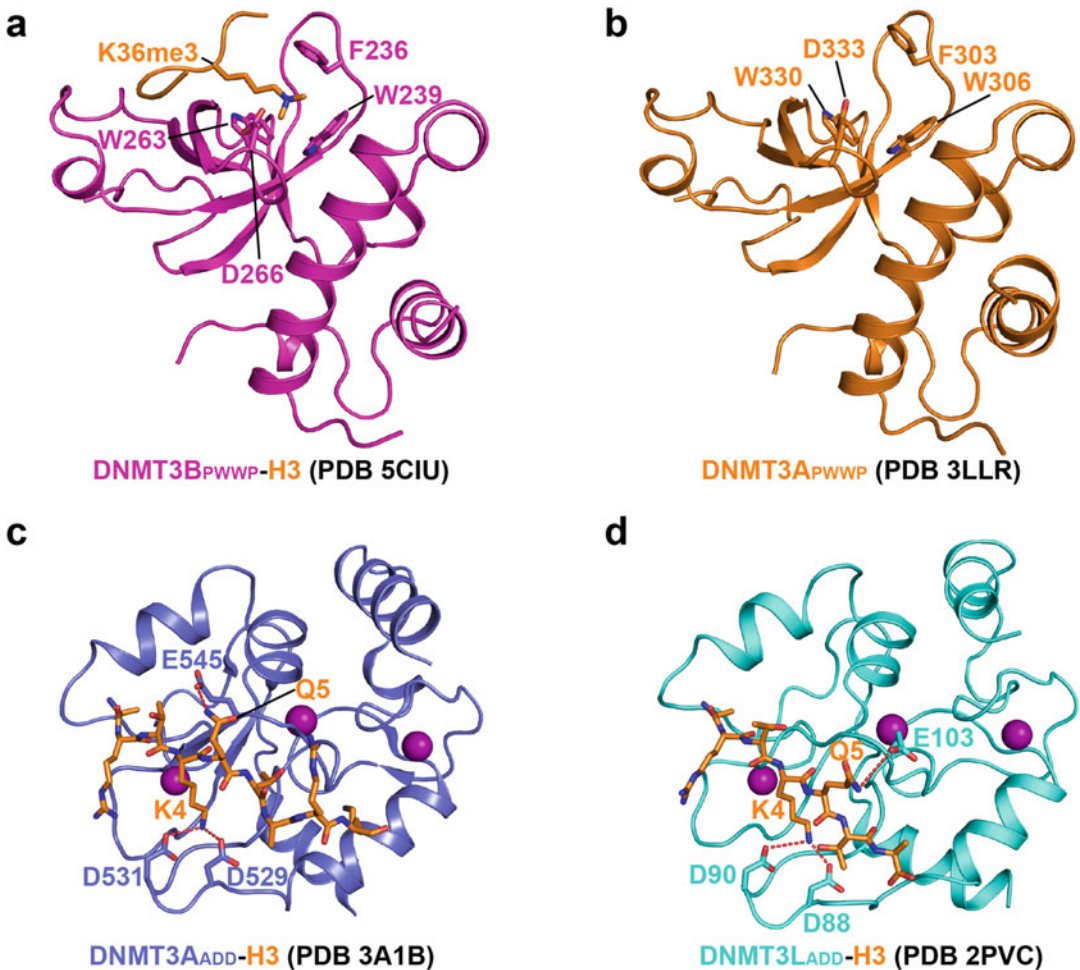


Fig. 3.2 Structures of the PWWP and ADD domains of Dnmt3s. (a) Ribbon diagram of the human DNMT3B PWWP domain bound to a H3K36me3 peptide (orange sticks), with the H3K36me3-binding cage shown in stick representation. (b) Ribbon diagram of the human DNMT3A PWWP domain, with the potential H3K36me2-binding cage shown in stick representation.

(c, d) Ribbon diagram of the ADD domain of human DNMT3A (PDB accession number 3A1B) (c) and human DNMT3L (PDB accession number 2PVC) (d) bound to a histone H3 peptide (orange sticks). The H3-interacting residues are conserved in Dnmt3l and Dnmt3a. The zinc ions are shown in sphere representation

chromosome instability in mouse ES cells (Noh et al. 2015), confirming the contribution of the ADD domain to Dnmt3 targeting and function.

Interestingly, the ADD domain of Dnmt3a is located at a position that inhibits the accession of substrate DNA to the catalytic domain (Guo et al. 2015). The binding of the N-terminal tail of histone H3 induces rearrangement of the ADD domain to change its position to the one that DNA can access. Enhancement of de novo

methylation at the chromatin region enriched in nucleosomes containing H3K4me0 reported previously (Li et al. 2011; Zhang et al. 2010) may be well explained by the conformational rearrangement of the ADD domain positioning (Guo et al. 2015) (Fig. 3.3). It will be important to determine whether or not other factors that are reported to interact with the ADD domain of Dnmt3a or Dnmt3b induce similar rearrangement of the enzyme to enhance de novo DNA methylation

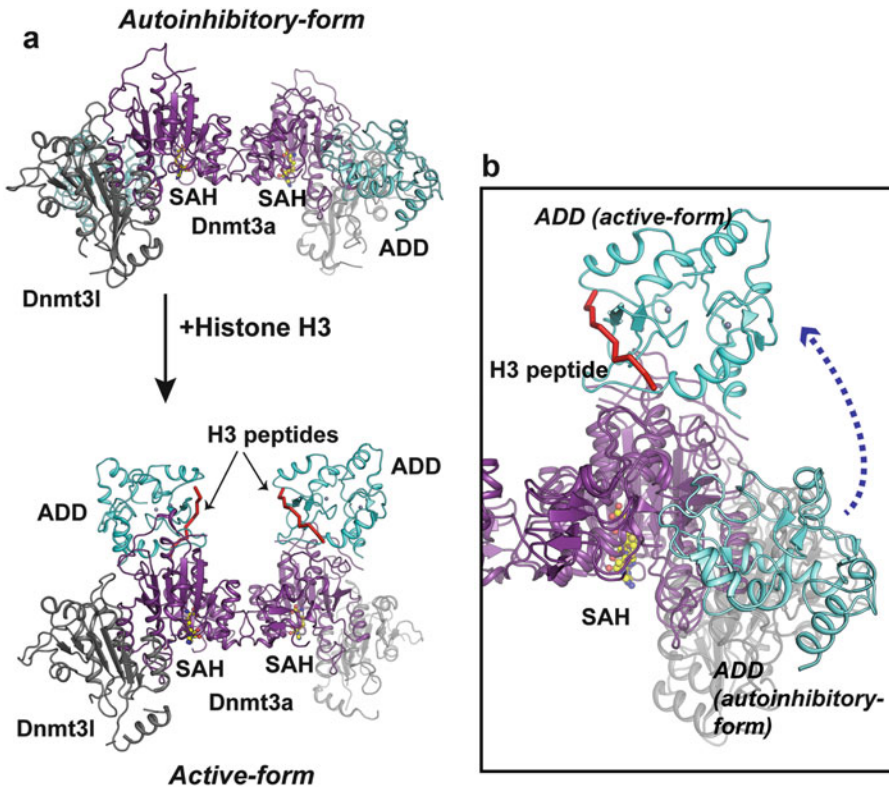


Fig. 3.3 Autoinhibition of DNMT3A by the ADD domain and histone H3 tail-induced activation of DNA methylation activity. (a) Ribbon illustrations of the structure of the complex of the catalytic domain with the ADD domain of Dnmt3a and the C-terminal half of Dnmt31 without (*upper*) or with (*lower*) a histone H3 tail. The catalytic domain is shown in magenta, the ADD domain in cyan, and the C-terminal region of Dnmt31 in gray. S-Adenosyl-L-homocysteine (SAH) is in yellow and the histone H3 tail in red. In the absence of a histone H3 tail,

substrate DNA cannot gain access to the catalytic center as the ADD domain is in a position that inhibits the DNA binding (autoinhibitory form; PDB accession number 4U7P). The addition of a histone H3 tail (*red*) drastically changes the position of the ADD domain to one that allows accession of DNA to the catalytic center (active form; PDB accession number 4U7T). (b) Superimposition of the active and autoinhibitory forms. The *dotted arrow* indicates the movement of the ADD domain from the bound form to the free form

activity, or rather reinforce ADD-mediated inhibition, as reported for methyl CpG binding protein 2 (MeCP2) (Rajavelu et al. 2018).

3.2.3 Catalytic Domain

In the catalytic domains of Dnmt3a and Dnmt3b, ten motifs characteristic of DNA-(cytosine C5)-methylation activity are conserved (Kumar et al. 1994). Dnmt3a and Dnmt3b interact through their catalytic domains with the C-terminal domain of Dnmt31, and this interaction enhances *de novo*

DNA methylation activity (Chen et al. 2005; Suetake et al. 2004). The crystal structure of the catalytic domain of Dnmt3a in complex with the C-terminal domain of Dnmt31 has been determined (Jia et al. 2007). It is a heterotetramer comprising two Dnmt3a molecules in the center and one Dnmt31 molecule at each edge (Fig. 3.3) (Jia et al. 2007; Jurkowska et al. 2011). The association of two Dnmt3a catalytic subunits in the center of the heterotetramer presumably increases the affinity for substrate DNA and is therefore crucial for DNA methylation activity. In the absence of Dnmt31, however, Dnmt3a

tends to polymerize using the same interaction surface as Dnmt3l. As the two interaction surfaces of Dnmt3a that cause polymerization contribute to its heterochromatin formation, it was proposed that the formation of the complex with Dnmt3l may promote releasing Dnmt3a from heterochromatin and facilitates Dnmt3a access to the substrate DNA (Jurkowska et al. 2011). It was also proposed that this inhibition of polymerization of Dnmt3a by Dnmt3l can be the underlying mechanism for the enhancement of DNA methylation activity of Dnmt3a (Jurkowska et al. 2011), especially in germ line cells to increase Dnmt3a availability and DNA methylation activity for the generation of global DNA methylation (Bourc'his et al. 2001; Hata et al. 2002; Kaneda et al. 2004). The association of Dnmt3a with Dnmt3l also serves to stabilize Dnmt3a in mouse ES cells (Veland et al. 2018).

The structures of the C-terminal domains of human DNMT3A-DNMT3L and DNMT3B-DNMT3L tetramers in complex with CpG DNA have been reported (Fig. 3.4a) (Anteneh et al. 2020; Gao et al. 2020b; Lin et al. 2020; Zhang et al. 2018). For the DNMT3A-DNMT3L-DNA complex, the two DNMT3A molecules bind to a single DNA duplex containing two separate CpG/ZpG ("Z" denotes zebularine) sites with 14bp interval, with the base of each zebularine flipped into the active site of DNMT3A, where it is stabilized via a covalent linkage with catalytic cysteine C710 and hydrogen-bonding interactions. Structural analysis of the DNMT3A-DNMT3L-DNA complexes reveals three major DNA-binding regions of DNMT3A: the catalytic loop (residues G707-K721), a loop (residues R831-F848) from the target recognition domain (TRD), and the DNMT3A/3A interface. The catalytic loop interacts with the DNA minor groove, the TRD loop interacts with the DNA major groove, while the DNMT3A/3A interface interacts with the DNA backbone of the segment bridging the two CpG sites. The substrate binding promotes the structural ordering of the TRD loop. Structural comparison of the CGT- and CGA-bound DNMT3A-DNMT3L complex further reveals that both the CpG recognition and the intramolecular interaction between the TRD loop

and the DNMT3A/3A interface occur in a context-dependent fashion: In the DNMT3A-DNMT3L-CGT DNA complex, the side chain of R882 forms hydrogen bonds with both the backbone and the side chain of S837, which stabilizes the TRD loop, wherein R836 forms a direct hydrogen bond with the CpG guanine (G6) (Fig. 3.4b). However, the hydrogen bonding interaction between R882 and the backbone of S837 is disrupted in the DNMT3A-DNMT3L-CGA DNA complex. Meanwhile, TRD loop residue N838 replaces R836 to form a hydrogen bond with G6, while R836 is repositioned toward the +1- and +2-flanking nucleotides for hydrogen-bonding and van der Waals contacts (Fig. 3.4c). No protein-DNA interaction was observed for the C-terminal domain of DNMT3L. The 14bp co-methylation spacing of the DNMT3A-DNMT3L complex was supported by a subsequent biochemical analysis (Gao et al. 2020a). However, its functional implication remains unclear.

The structures of human DNMT3B-DNMT3L-DNA complexes are highly similar to those of the DNMT3A-DNMT3L-DNA complexes (Fig. 3.4a). Nevertheless, the catalytic loop, the TRD loop, and the DNMT3B/3B interface all exhibit subtle differences in DNA contacts. First, the central interface of both catalytically active subunits engages in fewer DNA contacts in the DNMT3B-DNMT3L-DNA complexes than it does in the DNMT3A-DNMT3L-DNA complexes. Second, unlike DNMT3A R882 which is hydrogen bonded to the backbone of the TRD loop in the CGT complex, the corresponding DNMT3B R823 points away from the TRD loop in both CGT and CGA complexes (Fig. 3.4d,e). Third, unlike DNMT3A which interacts with the CpG site via R836 in the CGT complex but N838 in the CGA complex, DNMT3B interacts with the CpG site via an asparagine (N779)-mediated hydrogen bond regardless of the CGT or CGA context. Finally, a side-chain hydrogen bond is formed between DNMT3B N656 and R661 in the DNMT3B-DNMT3L-DNA complexes but not in the corresponding sites in the DNMT3A-DNMT3L-DNA complexes, due to the substitution of

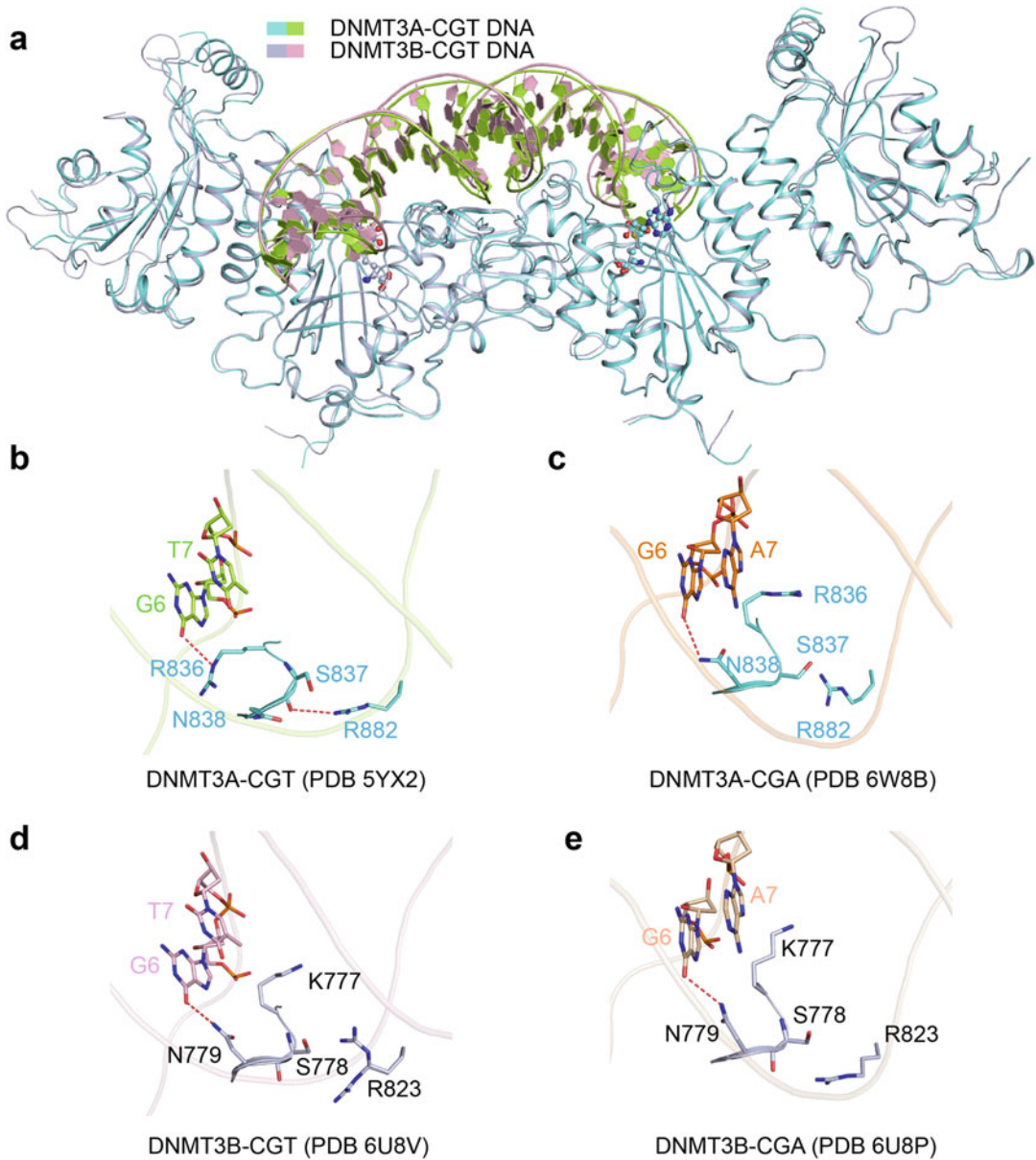


Fig. 3.4 Dnmt3a and Dnmt3b show similar but distinct substrate recognition mechanisms. **(a)** Structural overlay of human DNMT3A and DNMT3B catalytic domains covalently bound to ZpGpT/ApCpG (denoted as CGT) DNA. The SAH molecules are shown in ball-and-stick representation. **(b, c)** Close-up view of the CpG-specific

interaction by human DNMT3A TRD-loop residues in the context of CGT **(b)** and CGA **(c)** motif. The hydrogen bonds are shown as dashed lines. **(d, e)** Close-up view of the CpG-specific interaction by human DNMT3B TRD-loop residues in the context of CGT **(d)** and CGA **(e)** motif

DNMT3B N656 with DNMT3A I715. Consistent with these structural observations, biochemical and cellular analyses revealed distinct CpG specificity and flanking sequence preferences between Dnmt3a and Dnmt3b (Gao et al. 2020b; Lee et al.

2017; Lin et al. 2002; Lister et al. 2009, 2013; Wienholz et al. 2010), providing an explanation to their overlapped but distinct functionality during development (Okano et al. 1999). For instance, the fact that Dnmt3b prefers a purine,

whereas Dnmt3a prefers a pyrimidine, in the +1-flanking site explains why Dnmt3b-associated ICF mutations lead to pronounced hypomethylation of satellite II repeat, which is enriched with the CGA motif (Prosser et al. 1986).

The activity of Dnmt3a is also regulated by isoform 3 of Dnmt3b (Dnmt3b3), an inactive form of Dnmt3b, in somatic cells (Duymich et al. 2016). The cryo-EM structure of Dnmt3a2 in complex with Dnmt3b3 and nucleosome reveals that the C-terminal domain of Dnmt3b3 associates with the catalytic domain of Dnmt3a in a similar manner as the Dnmt3a-Dnmt3l complex (Xu et al. 2020). Furthermore, an interaction was identified between Dnmt3b3 and the acidic patch of the nucleosome, which regulates the Dnmt3a2-mediated DNA methylation in cells (Xu et al. 2020).

3.2.4 Functions of Other Regions

An N-terminal sequence upstream of the PWWP domain, present in Dnmt3a1 but not in the Dnmt3a2 isoform, strongly binds to DNA. This contributes to the DNA methylation activity and localization of the enzyme in nuclei (Suetake et al. 2011). As mentioned earlier, this region also contains a UDR domain that binds to H2AK119ub, which mediates the recruitment of Dnmt3a1 to H2AK119Ub-decorated genomic regions (Weinberg et al. 2021). The N-terminal sequence of Dnmt3b, which exhibits no homology with that of Dnmt3a, binds to centromere protein C (CENP-C). CENP-C is a constitutive centromere component and is necessary for mitosis. It was proposed that CENP-C recruits Dnmt3b to both centromeric and pericentromeric satellite repeats to methylate these regions (Gopalakrishnan et al. 2009). Moreover, it was reported that an Arg residue in the N-terminal region of Dnmt3a undergoes citrullination by peptidylarginine deiminase 4 (PAD4), which stabilizes Dnmt3a and increases the DNA methylation level of the promoter of the *p21* gene (Deplus et al. 2014). Moreover, Dnmt3b binds to NEDD8 (neuronal precursor cell-expressed developmentally down-regulated protein 8),

which is a small ubiquitin-like protein, through the region between the ADD and catalytic domains. NEDD8-modified Cullin 4A (CUL4A), which is essential for repressive chromatin formation, binds to Dnmt3b as well (Shamay et al. 2010).

3.2.5 Factors That Guide Dnmt3 to the Regions to Be Methylated

There have been several reports on the factors bringing Dnmt3 enzymes to specific sequences such as gene promoters. This mechanism is supported by the observation that a short DNA sequence (methylation-determining region, MDR) can determine the DNA methylation state (Lienert et al. 2011). Sequence-specific DNA-binding proteins may recognize such a sequence. For example, Dnmt3a binds to the corepressor complex of PR48 (regulatory subunit of protein phosphatase 2A)/HDAC1 (histone deacetylase 1) or proto-oncogene c-Myc through the ADD domain (Brenner et al. 2005; Fuks et al. 2001). Dnmt3b is reported to be tethered to the centromeric and pericentromeric heterochromatin regions through interaction with CENP-C to methylate the regions (Gopalakrishnan et al. 2009). Both Dnmt3a and Dnmt3b cooperate with EVI1 (oncogene product) to bind and methylate the expression-controlling region of miRNA 124-3 (Senyuk et al. 2011). Moreover, it was reported that noncoding RNA is involved in the targeting of Dnmt3b to de novo methylation sites. Promoter-associated RNA (pRNA), which binds the promoter of rRNA coding genes and forms a DNA/RNA triplex, recruits Dnmt3b to its target regions (Schmitz et al. 2010). However, it was also reported that the DNA/RNA heteroduplex rather inhibits the de novo methylation activities of both Dnmt3a and Dnmt3b in vitro (Ross et al. 2010).

In addition to the direct interaction with a DNA-binding protein or RNA, indirect interaction with the factors that bind to sequence-specific DNA-binding proteins has been reported. The Krüppel-associated box (KRAB) zinc finger

protein family, which determines target regions for methylation, comprising more than 300 genes (Liu et al. 2013), is an example. ZFP57, a KRAB zinc finger protein, binds to DNA in a sequence-specific manner and plays crucial roles in the establishment and maintenance of the methylation of imprinted genes through interaction with Tripartite motif containing 28 (Trim28, a.k.a. KAP1 or TIF1 β) (Quenneville et al. 2011, 2012). Trim28 interacts with Dnmt3a, Dnmt3b, and Dnmt1 (Zuo et al. 2012) and acts as a scaffold to guide Dnmts to a variety of target sequences utilizing sequence-specific binding of KRAB zinc finger proteins. As a similar example, NEDD8, which is a ubiquitin-like small protein modifier, acts as a tag in guiding Dnmt3b to NEDDylated proteins (Shamay et al. 2010). The main target of NEDDylation is Cullin, which plays a role in heterochromatin formation.

However, the recruitment of Dnmt3a to specific genomic regions does not always introduce DNA methylation. Although Dnmt3a is recruited to a target sequence by Ezh2 (enhancer of zeste homolog 2), a component of polycomb repressive complex 2 (PRC2) (Rush et al. 2009); MBD3 (methyl-CpG binding domain protein 3), an intrinsic component of corepressor complex NuRD (nucleosome remodeling deacetylase); Brg1 (Brahma-related gene-1), an ATPase subunit of Swi/Snf chromatin remodeling factor (Datta et al. 2005); or p53 (Wang et al. 2005), this recruitment does not affect the DNA methylation state of the target regions.

3.2.6 Correlation Between de novo DNA Methylation and Histone Modifications

The histone tail modifications directly recruit de novo-type Dnmt3a or Dnmt3b to the site of DNA methylation. As described above, the PWWP domain of Dnmt3a recognizes H3K36me2/H3K36me3 to enhance the DNA methylation activity (Dhayalan et al. 2010; Weinberg et al. 2019), and the ADD domain binds H3K4me0 (Li et al. 2011; Otani et al. 2009) to enhance the

DNA methylation activity (Li et al. 2011). The histone H3 tail with K4me3 inhibits DNA methylation by Dnmt3a (Li et al. 2011; Zhang et al. 2010), protecting H3K4me3-rich regions from DNA methylation. Dnmt3l, a member of the Dnmt3 family with no methylation activity, also contains an ADD domain and recognizes H3K4me0 (Ooi et al. 2007), as described above. H3K4me0 recruits and activates the Dnmt3a and Dnmt3l de novo methyltransferase complex to methylate the genome. In addition, the PWWP domains of Dnmt3a and Dnmt3b are reported to be a motif for DNA binding (Purdy et al. 2010; Qiu et al. 2002) and bringing Dnmt3a or Dnmt3b to heterochromatin (Chen et al. 2004; Ge et al. 2004). Thus, the PWWP in the amino-terminal half of Dnmt3a or Dnmt3b is one of the determinants of methylation-site targeting. Trim28, which is reported to interact directly with Dnmt3a (Zuo et al. 2012), also interacts with Setdb1, a histone H3K9 methyltransferase, and HP1 (Matsui et al. 2010), which recognizes H3K9me2/3.

3.3 Enzymes Responsible for the Maintenance of DNA Methylation Patterns

Dnmt1 is mainly responsible for maintaining DNA methylation patterns during replication or after DNA damage repair. Dnmt1 is a large molecule, comprising ~1,600 amino acid residues. Dnmt1 is composed of several domains: the N-terminal independently folded domain (NTD), replication foci-targeting sequence (RFTS) domain, CXXC domain, two bromo-adjacent-homology (BAH1 and BAH2) domains, and the catalytic domain (Fig. 3.1b). The domains are folded almost independently and interact with each other to form a functional DNA methyltransferase. The three-dimensional structures of mouse and human Dnmt1 with all the domains except for the NTD have been reported (Takeshita et al. 2011; Zhang et al. 2015).

3.3.1 NTD

The NTD of mouse Dnmt1 comprising amino acids (aa) 1–248 folds independently (Suetake et al. 2006). This domain functions as a binding platform for the factors that regulate the Dnmt1 function. The 1–118 aa sequence in the NTD, which is a typical coiled-coil structure and is lacking in the oocyte-specific Dnmt1 isoform (Gaudet et al. 1998; Mertineit et al. 1998), binds Dnmt1 associated protein 1 (DMAP1), which is a factor that represses transcription by cooperating with histone deacetylase HDAC2. DMAP1 binds to Dnmt1 at replication foci to assist the maintenance of the heterochromatin state as well (Rountree et al. 2000).

Proliferating cell nuclear antigen (PCNA), which binds DNA polymerase δ and other factors related to replication, is a prerequisite factor for replication. PCNA binds to the 160–178 aa sequence of mouse Dnmt1 (Chuang et al. 1997; Jimenji et al. 2019). The binding helps Dnmt1 maintain the methylation profile of the daughter DNA (Chuang et al. 1997) and recruits Dnmt1 to replication foci at the early and middle stages of the S-phase (Egger et al. 2006; Schermelleh et al. 2007). Therefore, it is thought to be involved in the replication-dependent DNA methylation process. However, the NTD domain containing the PCNA-binding motif is dispensable for the maintenance of the differentially methylated regions (DMRs) of imprinted genes, at least in ES cells (Garvilles et al. 2015). The cell-cycle regulating Rb protein is also reported to bind to the NTD (Robertson et al. 2000).

Interestingly, many epigenetic factors that may contribute to the formation and maintenance of heterochromatin are reported to bind to the NTD. De novo-type DNA methyltransferases Dnmt3a and Dnmt3b (Kim et al. 2002), heterochromatin-binding protein beta (HP1 beta) that selectively recognizes H3K9me_{2/3} (Fuks et al. 2003), and G9a that specifically methylates H3K9 (Esteve et al. 2006) bind to the NTD. All these interacting factors are related to the formation of heterochromatin, indicating that maintenance-type DNA methyltransferase Dnmt1 is tightly linked to histone methylation modification.

Although its function is not known, the NTD binds to cyclin-dependent kinase-like 5 (CDKL5) (Kameshita et al. 2008) and casein kinase (Sugiyama et al. 2010) and undergoes phosphorylation. The CDKL5 is reported to be a causative kinase for Rett syndrome. Rett syndrome is known to be caused mainly by a mutation in the *MeCP2* gene, of which the translation product specifically binds to methylated DNA and is a component of the corepressor complex. An impairment of the interaction between Dnmt1 and CDKL5 may contribute to the pathogenic process of Rett syndrome (Kameshita et al. 2008). Casein kinase 1 also interacts with the NTD. Phosphorylation with casein kinase 1 inhibits the DNA-binding activity of the NTD (Sugiyama et al. 2010). The function of the N-terminal region, which is a platform for the regulatory factors of Dnmt1, also seems to be regulated by different types of kinases (Esteve et al. 2011; Lavoie et al. 2011; Lavoie and St-Pierre 2011).

In addition, the NTD contains the DNA-binding 119–197 aa sequence, which overlaps with the PCNA-binding motif. The sequence contains an AT-hook-like motif and binds to the minor groove of AT-rich DNA. The DNA binding competes with the PCNA binding. Arg 133 and 136 in the sequence are crucial for the DNA-binding activity (Suetake et al. 2006). It has been proposed that this DNA-binding activity of the N-terminal domain contributes to the localization of Dnmt1 to AT-rich genome regions such as *Line1*, satellite, and the promoter of tissue-specific silent genes to maintain the fully methylated state of the repaired region that is hemimethylated (Suetake et al. 2006).

After the NTD, a flexible linker follows. Partial digestion with proteases can release the NTD 1–248 aa and the C-terminal part 291–1620 aa sequences (Suetake et al. 2006). According to the crystal structure of mouse Dnmt1 291–1620 aa, the structure of the RFTS domain has been determined after Pro 357 (Takeshita et al. 2011). The sequence starting from 249 to 356 aa seems to be a flexible region lacking an ordered structure. It has been reported that deletion of this region from Dnmt1 decreases maintenance methylation of the

genome (Borowczyk et al. 2009). However, it has recently been reported that even with deletion of the entire NTD including this region, Dnmt1 is fully active as a maintenance methyltransferase, at least in ES cells (Garvilles et al. 2015). The 1–353 sequence, which contains the NTD and the linker, binds to un-methylated DNA with CpG (Fatemi et al. 2001). However, as described above, the NTD also contains a DNA-binding domain, which exhibits a preference not for the CpG sequence but for an AT-rich (Suetake et al. 2006). The function of this linker is ambiguous at this moment.

3.3.2 RFTS Domain

The RFTS domain follows the NTD. This domain is necessary for Dnmt1 localization at the replication region at the late S-phase (Leonhardt et al. 1992). This recruitment depends on the tethering of Uhrf1 (ubiquitin-like with PHD and ring finger domains 1) to the hemimethylated DNA that appears after replication, and it is a prerequisite event for the replication-dependent maintenance of DNA methylation (Bostick et al. 2007; Sharif et al. 2007). Uhrf1 selectively binds to hemimethylated DNA through the SET and RING-associated (SRA) domain (Arita et al. 2008; Avvakumov et al. 2008; Hashimoto et al. 2008), to which the RFTS domain of Dnmt1 directly binds (Bashtrykov et al. 2014a; Berkyurek et al. 2014). Direct interaction of the RFTS domain with the SRA domain accelerates the hemimethylated DNA accession to the catalytic center. The SRA of Uhrf1 and Dnmt1 cannot bind to the same CpG site at the same time due to steric hindrance (Arita et al. 2008; Song et al. 2012). This clearly indicates that there must be a mechanism to hand the hemimethylated CpG from the SRA domain over to the catalytic center of Dnmt1, which may be involved in the direct interaction between the RFTS and SRA domains. How the hemimethylated CPG is transferred from the SRA domain to the catalytic center of Dnmt1 remains unclear.

The structure of the human RFTS domain itself has been elucidated (Syeda et al. 2011),

exhibiting a two-lobe fold that is almost identical to that in the catalytically active mouse Dnmt1 (Takeshita et al. 2011) and human DNMT1 (Zhang et al. 2015). The position of the RFTS domain in the catalytically active Dnmt1 is intriguing. Since the RFTS domain is inserted into the catalytic pocket, a substrate DNA cannot gain access to the catalytic center due to steric hindrance. The position of the RFTS domain is stabilized by hydrogen bonds between the RFTS and catalytic domains. When the substrate DNA is short, DNA methylation activity is inhibited due to the positioning of the RFTS domain (Bashtrykov et al. 2014b; Berkyurek et al. 2014; Syeda et al. 2011). Surprisingly, even if the RFTS domain occupies the catalytic pocket, Dnmt1 can methylate DNA when it is longer than 12 bp and a length of about 30 bp is necessary for its full activity (Berkyurek et al. 2014). When the substrate DNA size is 12 bp, which is exactly the size that fits into the catalytic pocket of Dnmt1 (Song et al. 2012), Dnmt1 cannot methylate substrate DNA. The DNA methylation activity of Dnmt1 that lacks the RFTS domain toward short hemimethylated DNA is efficiently inhibited by ectopically added RFTS domain (Berkyurek et al. 2014; Syeda et al. 2011). Since the full DNA methylation activity is acquired when the substrate DNA is longer than 30 bp, the catalytic domain of Dnmt1 may increase the DNA-binding affinity by two DNA-binding sites to trigger the removal of the RFTS domain from the catalytic pocket.

Amino acid residues Lys 23 (Nishiyama et al. 2013), Lys 14 and Lys18 of histone H3 (Ishiyama et al. 2017; Qin et al. 2015) are reported to be ubiquitylated. These modifications cooperate with the H3K9me3 mark to interact with the RFTS domain, thereby modulating maintenance methylation by Dnmt1 (Fig. 3.5a) (Ishiyama et al. 2017; Ren et al. 2020). The ubiquitin molecules bind to the N-terminal subdomain of the RFTS domain, while the H3 residues occupy the cleft between the N- and C-lobe, through eviction of the linker C-terminal to the RFTS domain (Fig. 3.5a). Binding to the H3K9me3 mark is further stabilized by the site corresponding to human DNMT1 W465, along with the residues

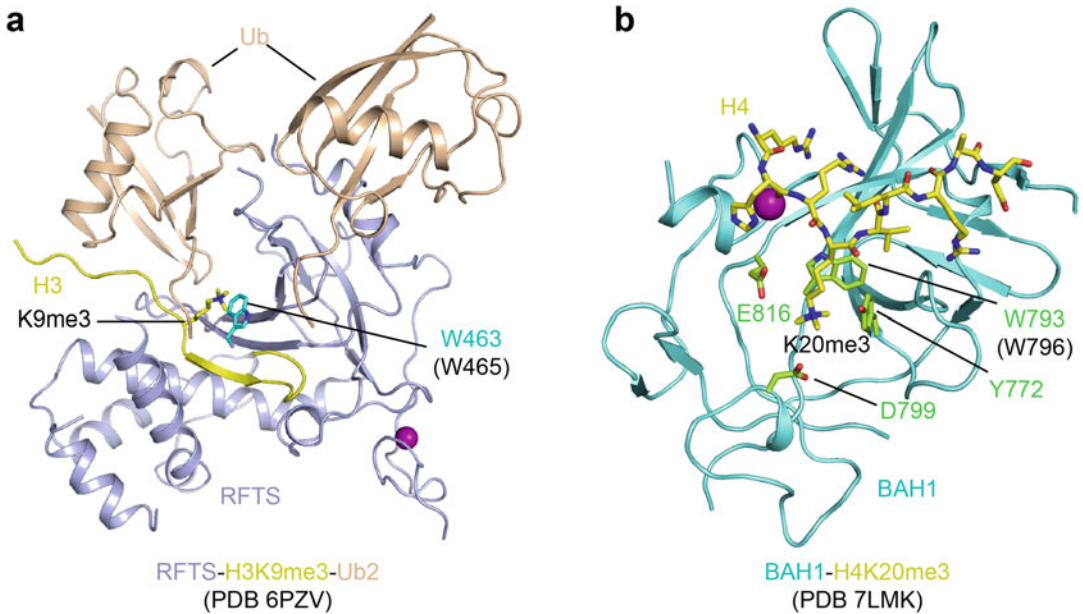


Fig. 3.5 Recognition of histone marks by Dnmt1 RFTS and ADD domains **(a)** Ribbon diagram of bovine Dnmt1 RFTS domain (slate) bound to a H3K9me3 peptide (yellow) and two ubiquitin molecules (wheat), with the H3K9me3-binding W463 shown in stick representation. The W463-corresponding site in human DNMT1 (W465)

is shown in parenthesis. **(b)** Ribbon diagram of bovine Dnmt1 BAH1 domain (cyan) bound to a H4K20me3 peptide (yellow), with the H4K20me3-binding cage residues shown in stick representation. The W793-corresponding site in human DNMT1 (W796) is shown in parenthesis. The zinc ions are shown as purple spheres in **(a)** and **(b)**

from one of the bound ubiquitin molecules (Fig. 3.5a). Introducing H3K9me3/H3 ubiquitylation binding-defective mutations to human DNMT1 led to a severe loss of genomic methylation and impairment of genome stabilization (Ren et al. 2020). In addition, Uhrf1 ubiquitylates lys 15 and lys 24 of PAF15 (PCNA-associated factor 15) during the early S-phase (Gonzalez-Magana et al. 2019; Nishiyama et al. 2020). The PAF15 protein with dual mono-ubiquitylation in turn recruits Dnmt1 to the replication foci via an interaction with the RFTS domain, an event important for the maintenance DNA methylation at early replicating domains (Gonzalez-Magana et al. 2019; Nishiyama et al. 2020).

Interestingly, the ring finger motif of Uhrf1, which is a prerequisite factor for replication-dependent maintenance methylation, is involved in the ubiquitylation of histones and DNMT1 as an E3 ligase (Du et al. 2010; Nishiyama et al.

2013; Qin et al. 2015). The tandem Tudor domain and the PHD finger of Uhrf1 recognize H3K9me3 and H3R2me0 (Arita et al. 2012), as well as a C-terminal poly-basic region of Uhrf1 (Fang et al. 2016; Gao et al. 2018; Gelato et al. 2014). Mutations within the tandem Tudor domain of UHRF1, which inhibit the recognition of H3K9me3, partly inhibit the maintenance DNA methylation (Rothbart et al. 2012; Zhao et al. 2016b), again indicating the cross-talk between DNA methylation and histone modification.

Following the RFTS domain, there are three residues, Phe 631, 634, and 635 (numbering based on mouse Dnmt1), in an alpha-helix structure interacting with Tyr 1243 and Phe 1246, which are adjacent to the PCQ loop in catalytic domain motif IV, of which the Cys residue covalently binds to the target cytosine at the sixth carbon. The interactions pull the PCQ loop toward the DNA-binding pocket (Takeshita et al. 2011).

3.3.3 CXXC

The CXXC domain contains two zinc atoms forming zinc finger motifs, which are known to bind DNA-containing un-methylated CpG. This motif is conserved among Dnmt1, mammalian trithorax-group protein, myeloid/lymphoid leukemia (MLL) (Cierpicki et al. 2010), CXXC-type zinc finger protein 1 (CXXC1) (Voo et al. 2000), methyl-CpG-binding protein 1 (MBD1) (Cross et al. 1997), and other proteins (Long et al. 2013). The CXXC domain of Dnmt1 contains two C4-type zinc fingers. The backbone structure of the CXXC domain does not change even when the RFTS domain is deleted ((Takeshita et al. 2011); Hashimoto et al., PDB accession number 3SWR), or the CXXC is bound to un-methylated DNA (Song et al. 2011).

The CXXC domain was initially proposed to be essential for the DNA methylation activity of Dnmt1 (Pradhan et al. 2008). However, this notion was later challenged by observations that removal of the CXXC domain does not substantially impair the Dnmt1 activity on DNA (Frauer et al. 2011; Song et al. 2011, 2012). When the RFTS domain is deleted, the autoinhibitory linker between the CXXC and BAH1 domains falls into the catalytic pocket, blocking the DNA from accessing the active site (Song et al. 2011). Song et al. proposed that binding of the CXXC domain to un-methylated DNA is a mechanism to inhibit its accession to the catalytic center of Dnmt1 and thus limits Dnmt1 from de novo methylation. This autoinhibitory mechanism cooperates with the intrinsic substrate specificity of the catalytic domain (Song et al. 2012) to modulate Dnmt1-mediated maintenance DNA methylation. This model predicts that deletion or mutation of the CXXC domain would increase de novo-type methylation activity. Although this hypothesis was supported by the observations for the RFTS-removed fragment (Song et al. 2011), full-length Dnmt1 with mutations in the CXXC domain did not show appreciable change in the specificity toward hemimethylated DNA in vitro ((Bashtykov et al. 2012); Suetake, unpublished observation). Reconstitution of

mouse *Dnmt1*^{-/-} ESCs with Dnmt1 containing CXXC mutations led to a similar genomic DNA methylation level to that with wild-type Dnmt1 (Frauer et al. 2011), likely due to the redundancy of Dnmt1 regulation. At present, the autoinhibition mechanism involving the CXXC domain to prevent de novo methylation (Song et al. 2011) awaits further investigation.

The autoinhibitory linker assumes a helical structure in DNA-free Dnmt1 (Takeshita et al. 2011; Zhang et al. 2015), but becomes extended in the RFTS-deleted Dnmt1 ((Song et al. 2011); Hashimoto et al., PDB accession number 3SWR). A mutation or deletion of this linker changes Dnmt1 into an extended conformation and enhances the DNA methylation activity toward 12 bp DNA (Zhang et al. 2015). Since such a short DNA cannot be methylated by Dnmt1 in the absence of the SRA domain of Uhrf1 (Berkyurek et al. 2014), it is reasonable to assume that this region plays a crucial role in the release of the RFTS domain from the catalytic pocket.

3.3.4 Two BAH Domains

The CXXC domain is followed by two tandem BAH domains. The BAH domains consist of a beta-sheet core and are functionally correlated to chromatin processes. The BAH domains from many other proteins, including the “remodels the structure of complex” component RSC2 (Chambers et al. 2013), Silent information regulator 3 (Sir3) (Armache et al. 2011; Arnaudo et al. 2013; Yang et al. 2013), the origin recognition complex subunit 1 (ORC1) (Kuo et al. 2012), BAH domain and coiled-coil containing 1 (BAHCC1) (Fan et al. 2020), BAH1 (Fan et al. 2021; Zhao et al. 2016a), SHORT LIFE (SHL) (Qian et al. 2018), EARLY BOLTING IN SHORT DAY (EBS) (Li et al. 2018b; Yang et al. 2018), and anti-silencing 1 (AS11)-IMMUNOPRECIPITATED PROTEIN 3 (AIPP3) (Zhang et al. 2020), interact with nucleosomes with various histone modifications. The two BAH domains of Dnmt1 are connected through an alpha-helix, which is dumbbell shaped

(Song et al. 2011; Takeshita et al. 2011). At the end of the BAH1 domain, just before the helix linker, there is a zinc finger motif which stabilizes DNMT1.

The BAH1 domain of Dnmt1 binds to histone H4K20me3 via a hydrophobic cage (Fig. 3.5b) (Ren et al. 2021). In the apo form of Dnmt1, this hydrophobic cage is shielded by the autoinhibitory linker. The interaction between the BAH1 domain and the H4K20me3 mark causes the displacement of the autoinhibitory linker, which in turn allosterically activates Dnmt1 (Ren et al. 2021). Single-molecule fluorescence resonance energy transfer (smFRET) analysis further indicated that the histone interactions of the BAH1 and RFTS domains both lead to enhanced conformational dynamics between the RFTS domain and the catalytic domain (Ren et al. 2021). Mutation of the hydrophobic cage residue W796 to alanine in human DNMT1 results in a H4K20me3 binding-defective but hyperactive enzyme, leading to DNA hypomethylation at the H4K20me3-decorated regions (e.g. Line1), but enhanced methylation at regions that lack H4K20me3 (Ren et al. 2021). Interestingly, the BAH1 W796A mutation can partially restore the DNA methylation that was reduced by the RFTS W465A mutation, raising a notion of the functional cooperation between the BAH1 and RFTS domains (Ren et al. 2021). Consistently, cells transfected with human DNMT1 containing W465A/W796A double mutation appear less sensitive to ionization radiation than those with DNMT1 W465A single mutation (Ren et al. 2021).

The BAH2 domain possesses a long loop protruding from its body, of which the distal end interacts with the TRD in the catalytic domain, and adjacent residues interact directly with the substrate DNA (Song et al. 2012). The function of BAH2 remains elusive, although evidence suggests that it may regulate Dnmt1-mediated de novo DNA methylation in vivo (Yarychivska et al. 2018a).

The KG-repeat between the BAH2 and the catalytic domain is conserved among species (Kimura et al. 1996; Tajima et al. 1995). This

repeat was observed to interact with ubiquitin-specific protease 7 (USP7), which is a deubiquitinating enzyme (Qin et al. 2011). This interaction increases DNA methylation activity possibly through stabilizing Dnmt1 (Cheng et al. 2015) or deubiquitylation of H3 (Yamaguchi et al. 2017). Acetylation of the Lys residues in the KG-repeat impairs the Dnmt1-USP7 interaction and promotes degradation of Dnmt1. On the other hand, a separate study showed that the Dnmt1-USP7 interaction unlikely plays a major role in the stabilization of Dnmt1 in somatic cells (Yarychivska et al. 2018b).

3.3.5 Catalytic Domain

Similar to other Dnmts, the ten motifs characteristic of DNA-(cytosine C5)-methyltransferases are conserved in the catalytic domain of Dnmt1. The DNA methylation mechanism of Dnmt1 is assumed to be identical to that of M.HhaI (Kumar et al. 1994). However, different from in M.HhaI (Cheng et al. 1993), the position of the side chain of Cys in the PCQ loop, which is expected to form a covalent bond with the sixth carbon of the target cytosine base (Song et al. 2012), turns toward target cytosine on the addition of methyl-group donor *S*-adenosyl-L-methionine (SAM) even in the absence of DNA (Takeshita et al. 2011). The side chain of the Cys faces away when SAM is catabolized to *S*-adenosyl-L-homocysteine (SAH) after the transfer of a methyl group in mouse Dnmt1. Interestingly, the side chain of the Cys in the PCQ loop of human DNMT1 does not completely face away even in the SAH-binding form (Zhang et al. 2015). The effect of this difference between the mouse and human enzymes remains to be determined.

The TRD in the catalytic domain of Dnmt1 is exceptionally long compared to those in other DNA methyltransferases. The TRD covers the hemimethylated DNA and holds the methylated cytosine through hydrophobic interactions (Fig. 3.6a) (Song et al. 2012). The target cytosine in the hemimethylated CpG is flipped out and inserts into the active site of Dnmt1 (Fig. 3.6a). According to the three-dimensional structure of

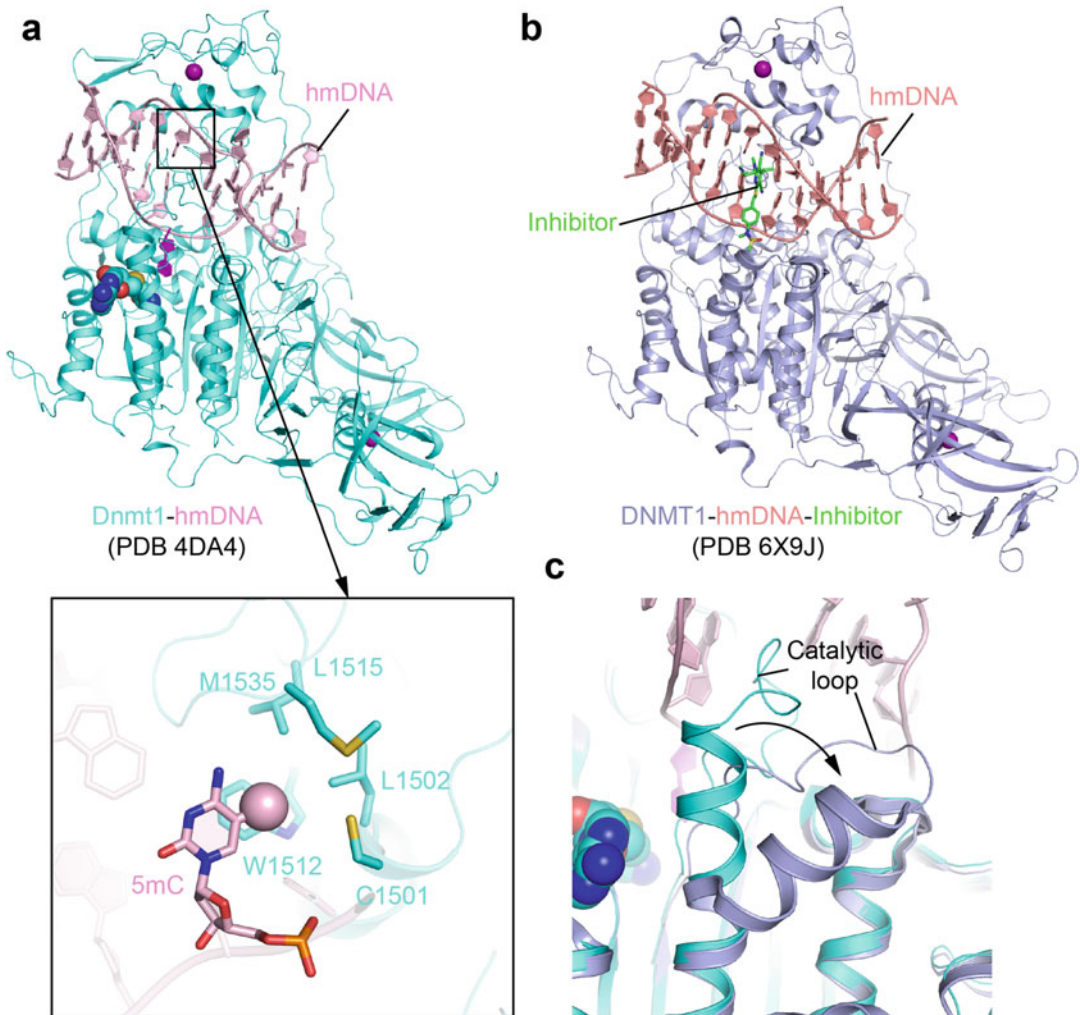


Fig. 3.6 Structure, mechanism and inhibition of Dnmt1-mediated maintenance DNA methylation. **(a)** Structure of mouse Dnmt1 C-terminal fragment (residues 731-1602) bound to hemimethylated CpG DNA (hmDNA), with flipped 5-fluorocytosine colored in purple. The van der Waals contacts between the 5-methyl group (sphere representation) of 5mC in the template strand and Dnmt1 residues are shown in expanded view. **(b)** Structure of

human DNMT1 C-terminal fragment (residues 729-1600) bound to hemimethylated CpG DNA and inhibitor GSK3830052. **(c)** Comparison of the active site conformation between the enzymatically active (PDB 4DA4) and inactive (PDB 6X9J) complex. The conformational shift of the helix C-terminal to the catalytic loop is indicated by arrow

the complex with hemimethylated DNA and the DNA methylation activity of the truncated Dnmt1, the recognition and selective methylation of hemimethylated DNA is at least in part underpinned by the catalytic domain (Bashtrykov et al. 2012; Song et al. 2012). A reversible small molecule inhibitor was identified to inhibit Dnmt1 activity through both DNA intercalation

and its interaction with Dnmt1 (Fig. 3.6b) (Pappalardi et al. 2021). The transition between catalytically active and inactive states of Dnmt1 is accompanied by a straight-to-kinked switch of the alpha-helix following the catalytic loop (Fig. 3.6c) (Pappalardi et al. 2021; Song et al. 2011, 2012; Ye et al. 2018).

In addition to its regulatory domains, the enzymatic activity of Dnmt1 is fine-tuned by the flanking sequence of the hemimethylated CpG sites (Adam et al. 2020). Structural comparison of Dnmt1 in complex with DNAs containing hemimethylated GCG, ACG, and CCG motifs (underlined are the CpG-flanking nucleotides) reveals distinct base-flanking mechanisms (Adam et al. 2020), which presumably causes the differential methylation activity of Dnmt1 on these substrates and impacts the dynamic landscape of DNA methylation in health and disease.

3.4 Cross-Talk Between De Novo-Type and Maintenance-Type DNA Methyltransferases

Establishment of DNA methylation patterns is mainly performed by de novo DNA methyltransferases, Dnmt3a and Dnmt3b, and their maintenance during replication is carried out by Dnmt1, as described above. However, it has been reported that Dnmt3a and/or Dnmt3b are also necessary for maintaining the methylation of repeat elements (Liang et al. 2002). In *Dnmt3a* and *Dnmt3b* double-knockout ES cells, DNA methylation gradually decreased during culture (Chen et al. 2003). A similar decrease in DNA methylation has been observed in mouse embryonic fibroblasts after *Dnmt3b* deletion (Dodge et al. 2005). These reports indicate that not only Dnmt1 but also de novo-type DNA methyltransferases Dnmt3a and/or Dnmt3b contribute to the maintenance DNA methylation. There has been a report that Dnmt3a and Dnmt3b interact with Dnmt1 at the NTD (Kim et al. 2002). It is unlikely, however, that Dnmt3a and Dnmt3b coexist with Dnmt1 at replication foci, since Dnmt1 is loaded at an early stage of replication, and Dnmt3a and Dnmt3b at a rather late stage of replication (Alabert et al. 2014). Therefore, the molecular mechanism of the cooperation with de novo-type Dnmts in maintenance DNA methylation remains to be determined.

As for the establishment of DNA methylation patterns, it was expected that Dnmt1 exhibits de

novo methylation activity in vivo (Christman et al. 1995). Actually, Dnmt1 exhibits a significant level of de novo-type DNA methylation activity in vitro (Fatemi et al. 2001; Vilkaitis et al. 2005) and ex vivo (Biniszkievicz et al. 2002; Haggerty et al. 2021; Li et al. 2018a; Takagi et al. 1995; Vertino et al. 1996; Wang et al. 2020; Yarychkivska et al. 2018a). In *Dnmt3a* and *Dnmt3b* knockout ES cells, ectopically introduced DNA (Lorincz et al. 2002) as well as endogenous regions (Arand et al. 2012) undergo de novo DNA methylation. Dnmt1 apparently favors de novo methylation near preexisting methylation sites (Arand et al. 2012; Vilkaitis et al. 2005). Therefore, although its physiological meaning is elusive, Dnmt1 also causes de novo DNA methylation in vivo. The cross-talk of de novo and maintenance DNA methylations is discussed in broader context in Jones and Liang (Jones and Liang 2009) and Jeltsch and Jurkowska (Jeltsch and Jurkowska 2014).

3.5 Conclusions and Perspective

Elucidation of the domain structures of Dnmts has provided important information in understanding the molecular mechanisms of DNA methylation. Indeed, the complexes of the ADD domain of Dnmt3a with histone H3, the PWWP domain of Dnmt3b with H3K36me3, the RFTS domain of Dnmt1 with H3K9me3 and H3 ubiquitylation, and the BAH1 domain of Dnmt1 with H4K20me3 illustrated their functions in the target recruitment and/or allosteric activation of the enzymes. Co-crystal structures of Dnmt3a with Dnmt3l and DNA and that of Dnmt1 with hemimethylated DNA have provided a clue to understand the DNA methylation mechanism. The domain rearrangement of Dnmt3a by histone H3 tail and occupation of the catalytic pocket of Dnmt1 by the RFTS domain have lifted the veils of DNA methylation tricks. In the near future, by utilizing the structural information, the biochemical approach with site-directed mutagenesis might provide further information in understanding molecular mechanisms of DNA methylation

regulation. To this end, we need more structural information including complexes with other factors.

In addition to the high-resolution crystal structures, NMR may possibly provide us with more dynamic structural information in solution, and analysis by single-particle cryogenic electron microscopy can be a powerful technology to analyze large complexes that may be involved in DNA methylation regulation in the chromatin environment.

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