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

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Abstract

In mammals, three 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, and in this chapter, the structures and functions of these domains are described.

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

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during replication in a cell lineage-dependent manner (Fig. 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 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). 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.

2 Enzymes Responsible for the Establishment of DNA Methylation Patterns

In mammals, two of the three DNA-(cytosine C5)-methyltransferases, Dnmt3a and Dnmt3b, which are encoded in distinct gene loci, are responsible for establishing the methylation patterns through their de novo-type DNA methylation activity (Okano et al. 1999; Aoki et al. 2001). Their domain arrangements are similar, each comprising a PWWP, ADD (Atrx-Dnmt3-Dnmt3l), and C-terminal catalytic domain (Fig. 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 (Fuks et al. 2001; Brenner et al. 2005; Otani et al. 2009). Dnmt3l, a homologue 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 DNA methylation (Bourc'his et al. 2001; Hata et al. 2002).

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 (Steca et al. 2000). The PWWP domain of Dnmt3b, comprising a beta-barrel structure with 5 beta-sheets 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 (Fig. 2a). The beta-barrel part of the PWWP domain is homologous to that of the SAND domain, which

is a DNA-binding motif, and the Tudor domain, which is generally a histone-binding motif. The PWWP domain of Dnmt3a also binds to DNA, though the affinity toward DNA is one order of magnitude lower compared to that of the PWWP domain of Dnmt3b (Purdy et al. 2010).

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 higher affinity of Dnmt3b to DNA than that of Dnmt3a could be the reason for the specific methylation of major satellites by Dnmt3b. 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 (Okano et al. 1999; Hansen et al. 1999). Additionally, the PWWP domain interacts with tri-methylated Lys 36 histone H3 (H3K36me3), and the binding is inhibited by the point mutation causing the ICF syndrome, indicating that the recognition of H3K36me3 is crucial for Dnmt3a to target chromatin (Dhayalan et al. 2010). On the other hand, it was also reported that the PWWP domain of Dnmt3b, but not that of Dnmt3a, is recruited to the H3K36me3-containing gene body for de novo methylation (Baubec et al. 2015).

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). It was also reported that the PWWP domain of Dnmt3a is involved in the interaction with thymine DNA glycosylase (TDG), and the interaction apparently inhibits the DNA methylation activity of Dnmt3a (Li et al. 2007b). Since the CpG sequences methylated by Dnmt3a and Dnmt3b are the target sites for hydroxylation by an oxygenase, the ten-eleven translocation (Tet) enzyme, in embryonic stem (ES) cells (Otani et al. 2013), and TDG are proposed to contribute to the final step of the removal of oxidized methylcytosines (He et al. 2011; Maiti and Drohat 2011); the interaction between TDG and Dnmt3a indicates their strong co-relationship.

2.2 ADD Domain

The plant homeodomain (PHD)-like Atrx-Dnmt3-Dnmt3l (ADD) domain, which is rich in Cys residues, is reported to bind 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), histone H4 symmetrically di-methylated at Arg 3 (H4R3me2s) (Zhao et al. 2009), 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. 2b) and possibly Dnmt3b as well (Dhayalan et al. 2011). 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 from DNA 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 3 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 also similar to that by that of Dnmt3l, 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. 2007a). Selective recognition of H3K4me0 by the ADD domains of Dnmt3a (Dnmt3b) and Dnmt3l may recruit de novo methyltransferases to the sites to be methylated.

In plants, a DNA methyltransferase named CMT (chromomethylase) of *Arabidopsis*, which methylates the CpHpG and/or CpHpH sequence, recognizes H3K9me with its chromodomain (Stroud et al. 2014). Similar to CMT, DNA methyltransferase Dim2 of *Neurospora crassa* also contains a chromodomain and is guided to H3K9me (Tamaru and Selker 2001). For this, CMT and Dim2 cause H3K9me-dependent methylation of DNA. Although mammalian Dnmts do not directly recognize H3K9me, they are reported to interact with heterochromatin protein 1 (HP1) (Fuks et al. 2003; Smallwood et al. 2007; El Gazzar et al. 2008), which specifically recognizes H3K9me2/3. For this, H3K9 methylation is proposed to be the cause and/or result of DNA methylation.

Interestingly, the ADD domain of Dnmt3a is located at a position that inhibits 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 (Zhang et al. 2010; Li et al. 2011) may be well explained by the conformational rearrangement of the ADD domain positioning (Guo et al. 2015) (Fig. 3). It will be important to determine whether or not the 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 activity.

2.3 Catalytic Domain

In the catalytic domains of Dnmt3a and Dnmt3b, ten motifs characteristic for DNA-(cytosine C5)-methylation activity are conserved (Kumar et al. 1994). Dnmt3a and Dnmt3b interact through their C-terminal domains with the C-terminal domain of Dnmt3l, and this interaction enhances de novo DNA methylation activity (Suetake et al. 2004; Chen et al. 2005). The crystal structure of the catalytic domain of the Dnmt3a in complex with the C-terminal half of Dnmt3l has been determined (Jia et al. 2007). It is a heterotetramer comprising two Dnmt3a molecules in the center and one Dnmt3l molecule at each edge (Fig. 3) (Jia et al. 2007; Jurkowska et al. 2011). The catalytic domain of Dnmt3a forms a dimer and this dimer formation is crucial for DNA methylation activity. The dimerization is expected to increase the affinity for substrate DNA as the DNA-binding site of Dnmt3a is rather small compared to that of bacterial M.HhaI. In the absence of Dnmt3l, 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 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 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). This is supported by the methylation property of Dnmt3a, which methylates periodical CpG with an 8 to 10 bp interval, this distance being estimated from the three-dimensional structure matching the DNA methylation position interval (Jia et al. 2007; Glass et al. 2009).

Interestingly, thymine DNA glycosylase (TDG), which is a T/G mismatch glycosylase, interacts with the PWWP or/and catalytic domains of Dnmt3a to modulate its DNA methylation activity (Li et al. 2006). Since methylated cytosine is converted to thymine through deamination, cytosine methylation is necessary to repair the methylation state after base excision repair. However, the physiological meaning of the interaction of Dnmt3a with TDG is rather complicated. Recently, 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 Tet enzymes (He et al. 2011; Maiti and Drohat 2011).

2.4 Functions of Other Regions

An N-terminal sequence upstream of the PWWP domain in Dnmt3a, which is missing 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). The N-terminal region containing this DNA-binding sequence is poor in secondary structure and folding is not induced even by binding to DNA (unpublished observation). The N-terminal sequence of Dnmt3b, which exhibits no homology with that of Dnmt3a, binds to 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). Recently, it was reported that an Arg residue in the N-terminal region undergoes citrullination by peptidylarginine deiminase 4 (PADI4), 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, which is a small ubiquitin-like protein, through the region between the ADD and catalytic domains. NEDD8-modified CUL4A, which is essential for repressive chromatin formation, binds to Dnmt3b as well (Shamay et al. 2010).

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/HDAC1 or proto-oncogene c-Myc through the ADD domain (Fuks et al. 2001; Brenner et al. 2005). 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). Recently, it was reported that noncoding RNA is involved in targeting of Dnmt3b to de novo methylation sites. 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 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 Trim28 (KAP1 or TIF1β) (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 KRAB zinc finger proteins. As a similar example, NEDD8, which is an 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, recruitment of Dnmt3a to specific genomic regions does not always introduce DNA methylation. Although Dnmt3a is recruited to a target sequence by Ezh2, a component of polycomb repressive complex 2 (PRC2) (Rush et al. 2009); MBD3, an intrinsic component of corepressor complex NuRD; Brg1, an ATPase subunit of Swi/Snf chromatin remodeling factor (Datta et al. 2005); or p53 (Wang et al. 2005), and this recruitment does not affect the DNA methylation state of the target regions.

2.6 Correlation Between de novo DNA Methylation and Histone Modifications

The PWWP domains of Dnmt3a and Dnmt3b are reported to be a motif for DNA binding (Qiu et al. 2002; Purdy et al. 2010) 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. It is not known yet, however, how the PWWP of Dnmt3a or Dnmt3b selectively recognizes heterochromatin. Such recruitment of Dnmt3a or Dnmt3b to specific regions is strongly correlated with the chromatin state or histone modifications. 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. Cullin and CENP-C, as described above, are heterochromatin finders. Many studies have shown that Dnmt3a recognizes the modified or unmodified histone tail. The PWWP domain of Dnmt3a recognizes H3K36me3 to enhance the DNA methylation activity (Dhayalan et al. 2010), and the ADD domain binds H3K4me0 (Otani et al. 2009; Li et al. 2011) to enhance the DNA methylation activity (Li et al. 2011). The histone H3 tail with K4me3 inhibits DNA methylation by Dnmt3a (Zhang et al. 2010; Li et al. 2011). Dnmt3l, a member of the Dnmt3 family with no methylation activity, also contains an ADD domain and recognizes H3K4me0

(Ooi et al. 2007). H3K4me0 recruits the Dnmt3a and Dnmt3l de novo methyltransferase complex to methylate the genome. In addition, symmetric di-methylation of Arg 3 of histone H4 (H4R3me2S) is reported to be the target of Dnmt3a via the ADD domain for DNA methylation (Zhao et al. 2009). The histone tail modifications directly recruit de novo-type Dnmt3a or Dnmt3b to the site of DNA methylation.

3 Enzymes Responsible for the Maintenance of DNA Methylation Patterns

Dnmt1 is mainly responsible for maintaining DNA methylation patterns during replication or after repair. Dnmt1 is a large molecule; mouse Dnmt1 comprises 1,620 amino acid residues. Dnmt1 is composed of several domains: the N-terminal independently folded domain (NTD), replication foci-targeting sequence (RFTS) domain, CXXC motif, two bromo adjacent homology (BAH1 and BAH2) domains, and the catalytic domain (Fig. 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).

3.1 NTD

The NTD of mouse Dnmt1 comprising amino acids (aa) 1–248 folds independently (Suetake et al. 2006). The domain binds many factors and thus functions as a 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 oocyte-type Dnmt1 (Mertineit et al. 1998; Gaudet 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 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. The binding helps Dnmt1 maintain the methylation profile of the daughter DNA (Chuang et al. 1997) and recruit Dnmt1 to replication foci at the early and middle stages of the S-phase (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 H3K9me2/3 (Fuks et al. 2003), and G9a that specifically methylates H3K9 (Estève 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 (Estève et al. 2011; Lavoie and St-Pierre 2011; Lavoie et al. 2011).

In addition, the NTD of mouse Dnmt1 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). We hypothesized 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 easily releases 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, the NTD contains a DNA-binding domain, which exhibits a preference not for the CpG sequence but for an AT-rich sequence (Suetake et al. 2006). The function of this linker is ambiguous at this moment.

3.2 RFTS Domain

The replication foci-targeting sequence (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 (Berkyurek et

al. 2014; Bashtrykov et al. 2014a). 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 must be involved in the direct interaction between the RFTS and SRA domains (Fig. 4).

The structure of the human RFTS domain itself has been elucidated (Syeda et al. 2011) and turned out to be almost identical to that in the catalytically active mouse Dnmt1 (Takeshita et al. 2011). 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 (Syeda et al. 2011; Berkyurek et al. 2014; Bashtrykov et al. 2014b). 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 (Syeda et al. 2011; Berkyurek et al. 2014). The fact that Dnmt1 forms a head-to-head dimer through interaction between the RFTS domain (Fellinger et al. 2009) may contribute to the substrate length dependence of the activity. 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 removal of the RFTS domain from the catalytic pocket.

Amino acid residues Lys 23 (Nishiyama et al. 2013) and Lys18 of histone H3 (Qin et al. 2015) are reported to be ubiquitylated. These modifications are necessary for maintenance methylation via the interaction with the RFTS domain. Interestingly, the ring finger motif of Uhrf1, which is a prerequisite factor for replication-dependent maintenance methylation, is involved in the ubiquitylation as an E3 ligase (Citterio et al. 2004). The tandem Tudor domain and the PHD finger of Uhrf1 recognize H3K9me3 and H3R2me0 (Arita et al. 2012), and the mutation that inhibits the recognition of H3Kme3 partly inhibits the maintenance DNA methylation (Rothbart et al. 2012) again indicating the cross talk between DNA methylation and histone modification.

Following the RFTS domain, there are three residues, Phe 631, 634, and 635, 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). Mutation of the residues decreases the DNA methylation activity (unpublished observation).

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 mammalian trithorax-group protein, myeloid/lymphoid leukemia (MLL) (Cierpicki et al. 2010), CXXC-type zinc finger protein 1 (CXXC1) (Voo et al. 2000), methylated DNA-binding protein 1 (MBD1) (Cross et al. 1997), and other proteins (Long et al. 2013), including Dnmt1. 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).

When the RFTS domain is deleted, the CXXC domain falls into the catalytic pocket, and the CXXC domain at this position binds to un-methylated DNA (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 protects from methylating un-methylated DNA. If this is the case, it is reasonable to expect that deletion or mutation of the CXXC domain, which prevents the accession of un-methylated DNA to the catalytic pocket, would increase de novo-type methylation activity. However, the specificity of Dnmt1 with mutations in the CXXC domain does not cause any reduction of the genomic DNA methylation level (Frauer et al. 2011) or the specificity toward hemimethylated DNA methylation activity (Bashtrykov et al. 2012; Suetake, unpublished observation). In addition, Song et al. have reported that even Dnmt1 with the CXXC domain deleted shows similar specificity toward a hemimethylated DNA substrate (Song et al. 2012). This strongly suggests that the CXXC domain binding to un-methylated DNA does not contribute to inhibition of the methylation of un-methylated DNA. The effect of the CXXC domain mutation on the DNA methylation activity of Dnmt1 is rather controversial as Pradhan et al. reported that the CXXC is necessary for DNA methylation activity (Pradhan et al. 2008). Therefore, at present, the auto-inhibition mechanism involving the CXXC domain to prevent de novo methylation proposed by Song et al. (Song et al. 2011) needs further investigation.

The next BAH1 domain is connected with the CXXC domain by an alpha-helix structure (Takeshita et al. 2011). This helix is destroyed in the RFTS-deleted Dnmt1 without a change in the CXXC domain structure (Song et al. 2011; Takeshita et al. 2011; Hashimoto et al., PDB accession number 3SWR). A mutation or deletion of the helix changes the 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 helix region plays a crucial role in the release of the RFTS domain from the catalytic pocket.

3.4 Two BAH Domains

The CXXC domain is followed by two tandem BAH domains. The BAH domains consisting of a beta-sheet core are functionally correlated to chromatin processes. The BAH domain of RSC2, which is a component of “remodels the structure of complex” (RSC) (Chambers et al. 2013), and that of Sir3 (Armache et al. 2011; Arnaudo et al. 2013; Yang et al. 2013)

interact with nucleosomes and that of ORC1, a subunit of the origin recognition complex (ORC), possesses a hydrophobic cage recognizing H4K20me2 (Kuo et al. 2012). However, the function of the two BAH domains of Dnmt1 remains unknown.

The two BAH domains of Dnmt1 are connected through an alpha-helix, which is dumbbell shaped (Takeshita et al. 2011; Song et al. 2011). At the end of the BAH1 domain, just before the helix linker, there is a zinc finger motif. The formation of this Zn-finger is necessary for a stable conformation of Dnmt1, as mutations of the involved Cys residues inhibit solubilization of the Dnmt1 protein (unpublished observation).

Interestingly, the first BAH domain (BAH1) possesses a hydrophobic cage, which is expected to recognize the methylated histone tail. Four out of six amino acid residues of the hydrophobic cage of the mouse ORC1 recognizing H4K20me2 are conserved in the BAH1 domain of Dnmt1 (Yang and Xu 2013). This may suggest that Dnmt1 interacts with the methylated histone tail in the nucleosome structure. Since the cage is masked by a long loop traversing toward the CXXC domain from the N-terminal end of the BAH1 domain, the methylation modification cannot gain access to the hydrophobic cage in this conformation (Takeshita et al. 2011). What the target of this cage structure is remains to be determined. The BAH2 domain possesses a long protruding loop from its body, of which the distal end interacts with the target recognition domain (TRD) in the catalytic domain, and adjacent residues interact directly with the substrate DNA (Song et al. 2012). Although the structure of the two BAH domains has been elucidated, their function remains elusive. Furthermore, no one has succeeded in preparing DNA methylation-active Dnmt1 without the two BAH domains suggesting that they play a crucial role in the enzyme folding or activity.

The KG-repeat between the BAH2 and catalytic domains is conserved among species (Tajima et al. 1995; Kimura et al. 1996). Until recently, this repeat was thought to be just a hinge providing flexibility to the N-terminal region and the catalytic domain. Recently, it was reported that the KG-repeat is involved in the interaction 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). Acetylation of the Lys residues in the KG-repeat impairs the Dnmt1-USP7 interaction and promotes degradation of Dnmt1.

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 (AdoMet) even in the absence of DNA (Takeshita et al. 2011) (Fig. 5a, c). The side chain of the Cys faces away when AdoMet is catabolized to *S*-adenosyl-L-homocysteine (AdoHcy) after the transfer of a methyl group in mouse Dnmt1 (Fig. 5b). Interestingly, the side chain of the Cys in the PCQ loop of human DNMT1 does not completely face away the side chain position even in the AdoHcy-binding

form (Zhang et al. 2015) (Fig. 5d). The effect of this difference between the mouse and human enzymes remains to be determined.

The target recognition domain (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 (Song et al. 2012). The target cytosine in the hemimethylated CpG is flipped out and is directly involved in the catalytic loop. According to the three-dimensional structure of the complex with hemimethylated DNA and the DNA methylation activity of the truncated Dnmt1, the recognition and selective methylation of hemimethylated DNA exist in the catalytic domain itself (Song et al. 2012; Bashtrykov et al. 2012).

4 Cross Talk Between De Novo- 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, 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). Ectopically overexpressed Dnmt1 causes de novo DNA methylation (Takagi et al. 1995; Vertino et al. 1996; Biniszkiewicz et al. 2002). 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 (Vilkaitis et al. 2005; Arand et al. 2012). Therefore, although the 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 (2009) and Jeltsch and Jurkowska (2014).

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 complex of the ADD domain of Dnmt3a with histone H3 and the PWWP domain of Dnmt3b with DNA illustrated their functions in the recruitment of the enzymes to specific sites. Co-crystal structures of Dnmt3a with Dnmt3l 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 advanced technology of single particle analysis by electron microscopy can be a powerful technology to analyze large complexes that may be involved in DNA methylation regulation.

Abbreviations

AdoHcy	*S*-Adenosyl-L-homocysteine
AdoMet	*S*-Adenosyl-L-methionine
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
SRA domain	The SET and RING-associated domain
TDG	Thymine DNA glycosylase
Tet enzyme	Ten-eleven translocation enzyme
TRD	The target recognition domain

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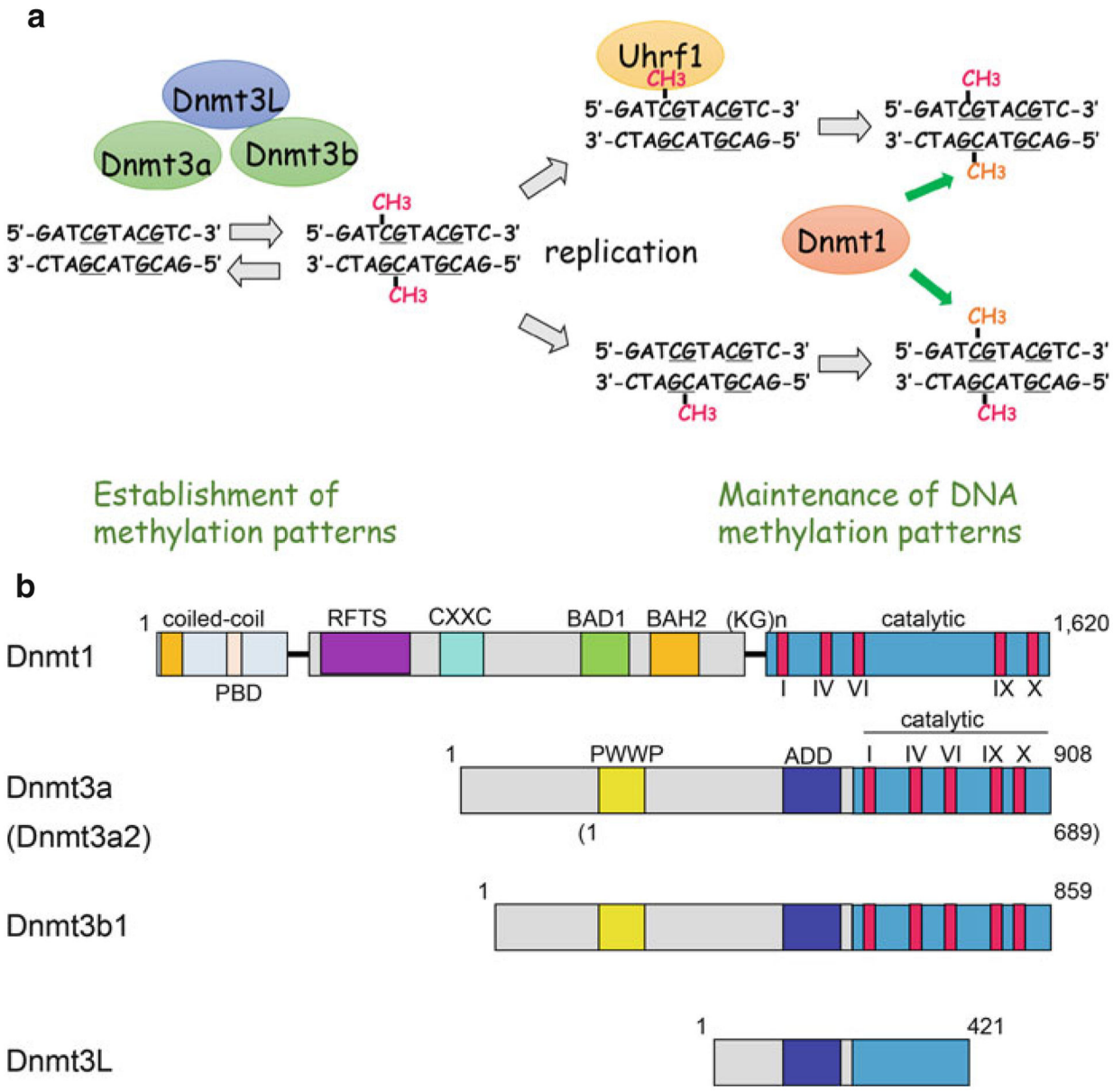

Fig. 1.

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

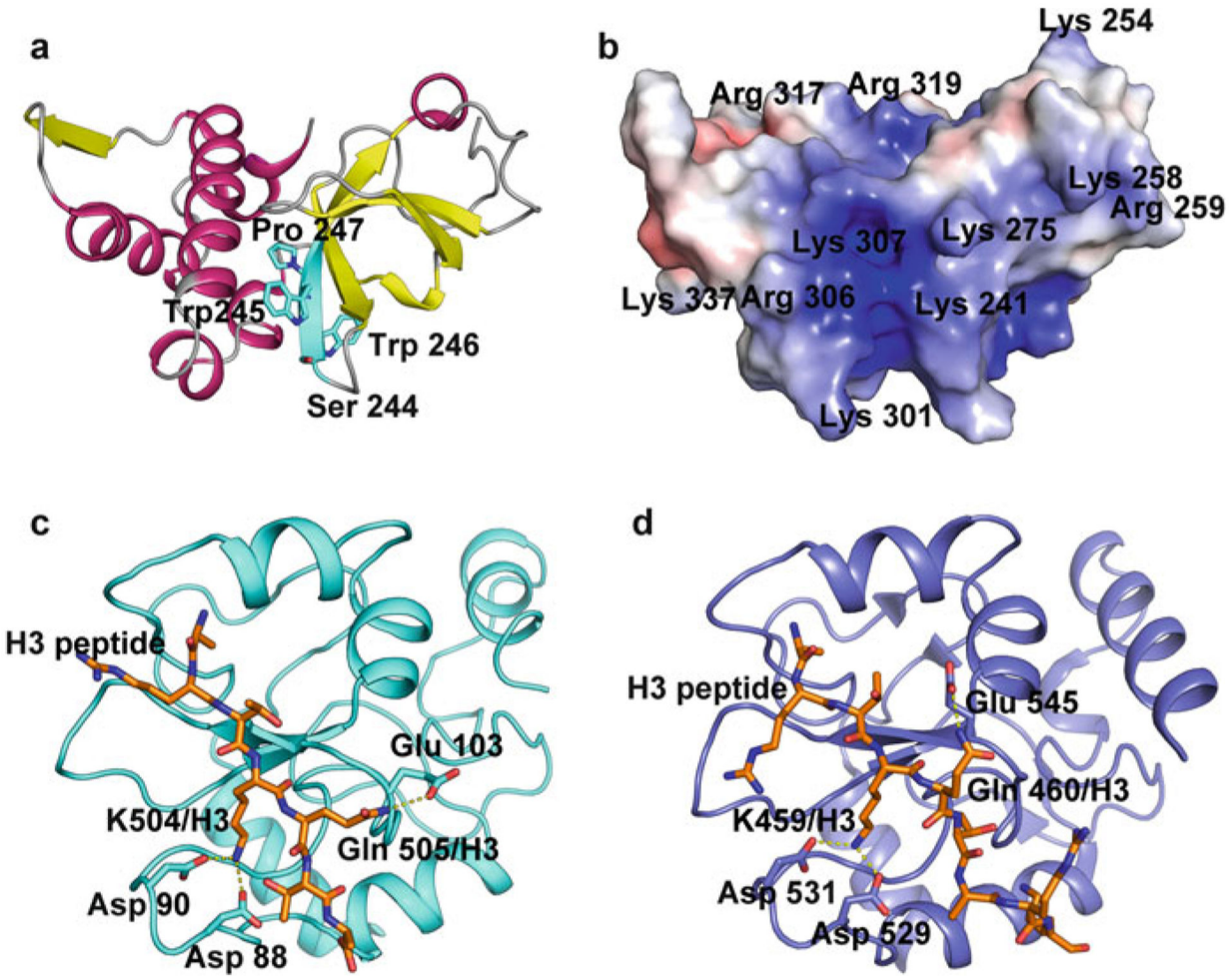

Fig. 2.

Structure of the PWWP domain of Dnmt3b. (**a**) Ribbon diagram of the PWWP domain, which is changed to SWWP in Dnmt3b (*left*), and the molecular surface with the charge distribution of the PWWP domain of Dnmt3b (*right*) are shown. Positively charged amino acids (Lys and Arg) are in *blue*, negatively charged ones (Glu and Asp) in *red*, and uncharged ones in *white*. The positions of Lys and Arg residues are indicated (PDB accession number 1KHC). (**b**) Ribbon diagram of the ADD domain of Dnmt3l (PDB accession number 2PVC) (*left*) and Dnmt3a (PDB accession number 3A1B) (*right*). Histone H3 peptides are in brown. The interacting amino acid residues with histone tail peptide are conserved in Dnmt3l and Dnmt3a

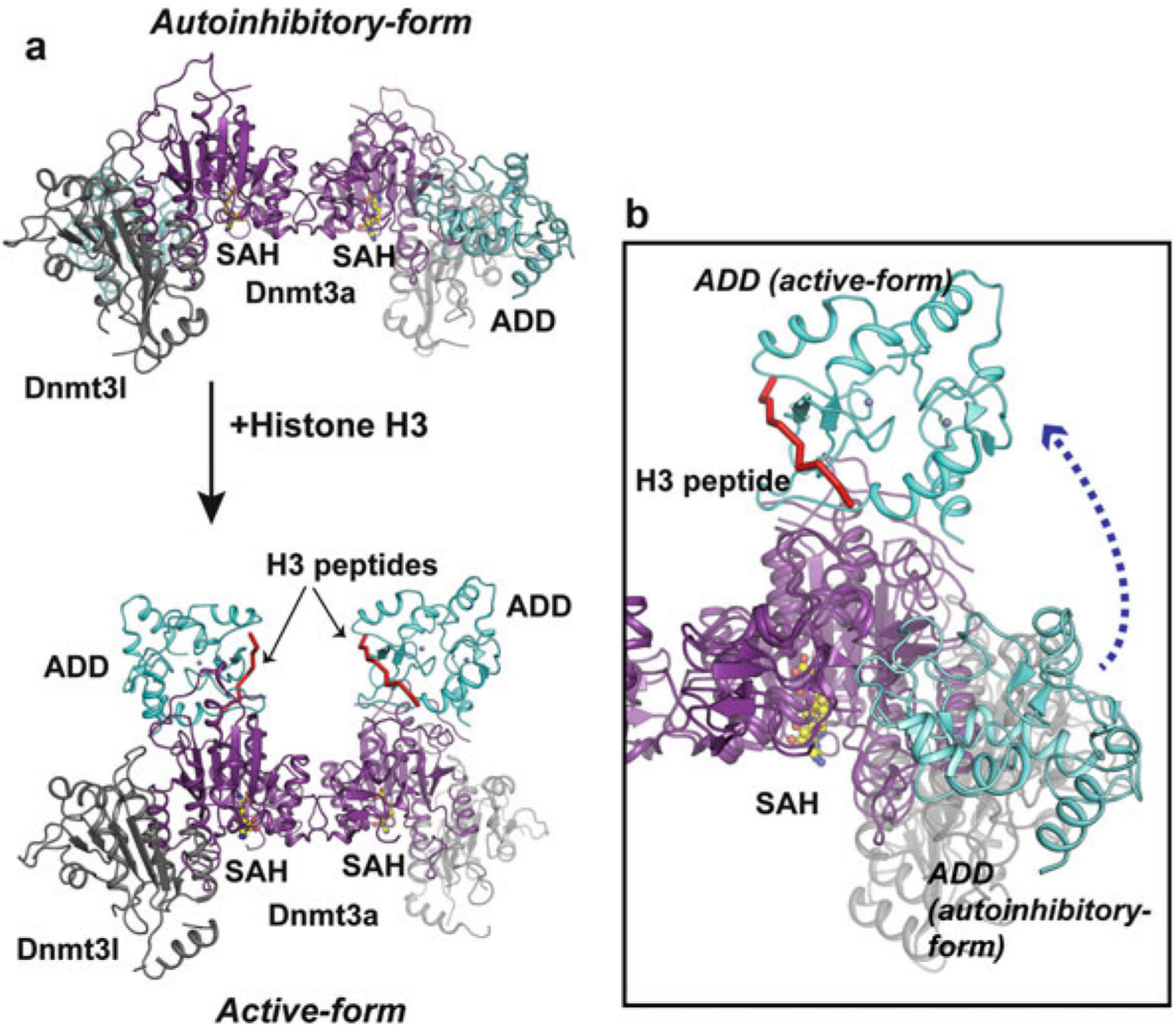

Fig. 3.

Auto-inhibition 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 Dnmt3l 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 Dnmt3l in gray. *S*-Adenosyl-L-homocysteine (AdoHcy) 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 (auto-inhibitory 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 auto-inhibitory forms. The *dotted arrow* indicates the movement of the ADD domain from the histone H3 tail free to the bound form

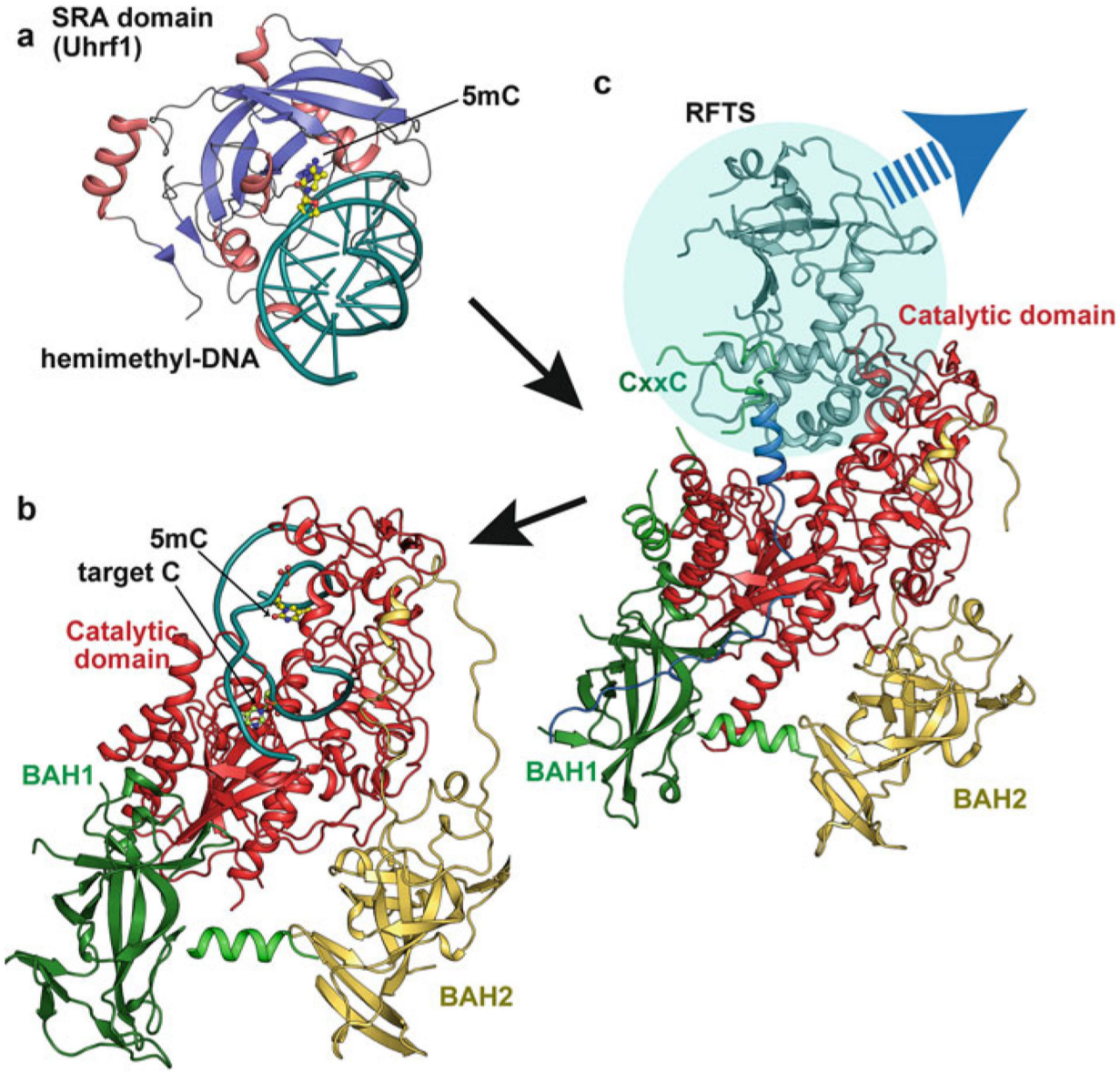

Fig. 4.

Transfer of hemimethylated DNA from Uhrf1 (SRA domain) to the catalytic center of Dnmt1. (**a**) Hemimethylated DNA, which appears just after the replication, is occupied by Uhrf1 (SRA domain) (PDB accession number 2ZKE). (**b**) Considering the reported structure of the Dnmt1 catalytic domain complex with hemimethylated DNA (PDB accession number 4DA4), SRA and Dnmt1 cannot recognize hemimethylated CpG at the same time. (**c**) Furthermore, the RFTS domain occupies the catalytic pocket of Dnmt1 (PDB accession number 3AV6). The SRA domain of Uhrf1 directly interacts with the RFTS domain of Dnmt1 to remove the domain from the catalytic pocket to allow hemimethylated DNA access to the catalytic center

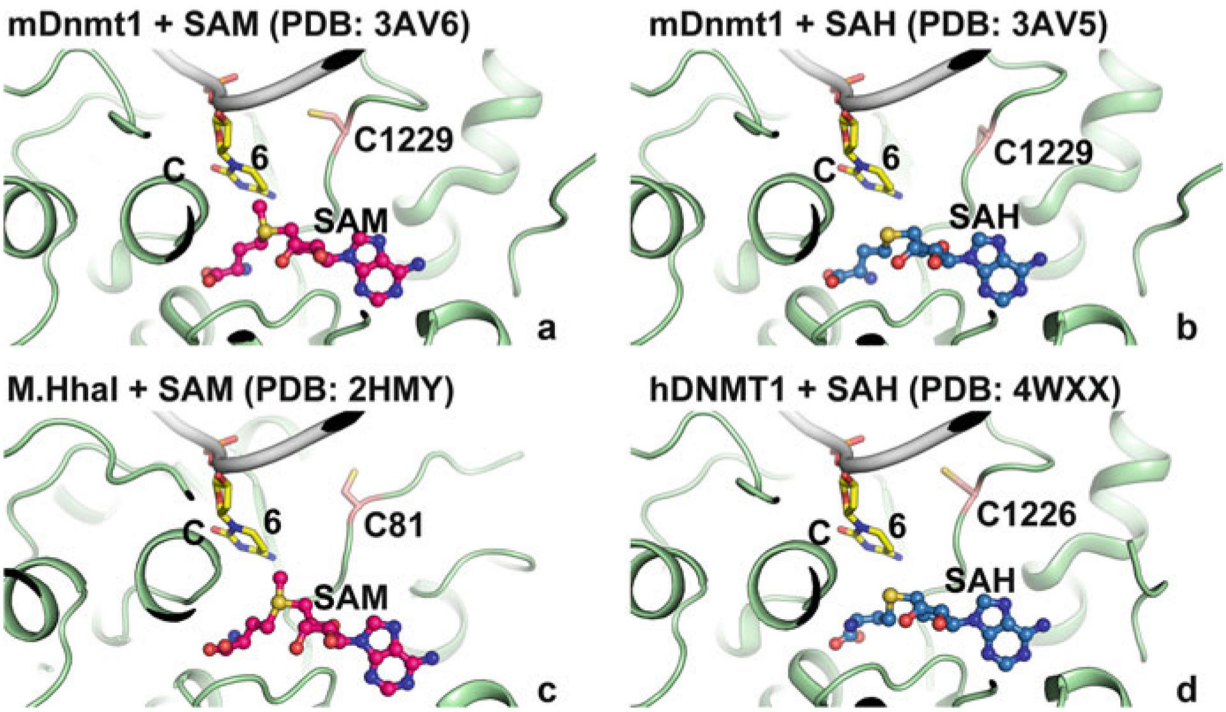

Fig. 5.

Positioning of Cys residues that covalently bind the sixth position of the target cytosines in mouse Dnmt1, human DNMT1, and M.HhaI. Cys 1229 in the PCQ loop of mouse Dnmt1 faces toward or away from the target cytosine in the presence of AdoMet or AdoHcy, respectively (**a**, **b**). On the contrary, in M.Hha1, Cys 81 in the PCQ loop faces away from the target cytosine even in the presence of AdoMet when DNA is not present (**c**). Different from mouse Dnmt1, in human DNMT1, C1226 in the PCQ loop still faces toward the target cytosine in the presence of AdoHcy (**d**)