DNA methyltransferases hitchhiking on chromatin

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DNA methyltransferases hitchhiking on chromatin

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Summary

DNA methylation is an epigenetic modification that plays a central regulatory role in various biological processes. Methyl groups are coupled to cytosines by the family of DNA methyltransferases (DNMTs), where DNMT1 is the main maintenance enzyme and the DNMT3 branch of the family is mostly responsible for *de novo* methylation. The regulation and function of DNA methylation are dependent on the genomic and chromatin context, such as binding sites for transcription factors or the presence of histone marks. Yet how local context, especially chromatin marks, influences the recruitment of the different DNMTs to their genomic target sites remains to be completely revealed. Elucidating the crosstalk between different histone modifications and DNA methylation, and their combined effect on the genome-wide epigenetic landscape, is of particular interest. Aberrant distribution of chromatin marks that guide DNMT activity or *DNMT* mutations that influence their correct recruitment to the genome have a profound impact on the deposition of DNA methylation, with consequences for genome function and gene activity. In this review, we describe the current state of knowledge on this topic and provide an overview on how chromatin marks can guide DNMT recruitment in healthy and diseased cells.

Keywords: *DNA methylation*, *epigenetics*, *chromatin*

Introduction

While the field of genetics studies heredity, in which genes that are encoded in the DNA represent the basic units of inheritance, the field of epigenetics (from the Greek ἐπί, meaning "above") studies those mechanisms that effectuate heritable changes without altering the underlying DNA sequence itself. Such changes are known as epigenetic modifications and exist in a variety of forms. The only epigenetic modification that takes place directly at the level of the nucleosides is DNA methylation, which comprises the coupling of a methyl group (CH_3) to DNA bases. Although other contexts are permissive, most methylation in vertebrates appears on the 5′ position of the pyrimidine ring of cytosine, resulting in 5-methylcytosine (5meC) [1]. Apart from a low prevalence of 5meC at CpA, CpT, and CpC sequences in certain cell types, mammalian cytosine methylation is generally found in the setting of a cytosine followed by a guanine on the same strand, constituting a self-complementary CpG dinucleotide [2, 3]. Due to the inherent mutability of 5meC the general occurrence of CpGs in the genome is low, with the exception of so-called CpG islands (CGIs), which are short regions of high CpG content that are commonly associated with gene promoters [4]. Whereas approximately 70% of CpG sites in the adult vertebrate genome are methylated, the majority of CGIs remain free from methylation [4]. CGI promoter methylation can regulate transcription of associated genes *in cis*, which is particularly important for X chromosome inactivation, genomic imprinting, and germline-specific genes [5–7]. In addition, DNA methylation has been shown to control the silencing of transposable elements [8]. Together, this demonstrates that cytosine methylation has a central regulatory function in a variety of biological processes.

DNA methylation is a dynamic process, which can be divided into three stages: establishment, maintenance, and removal. The DNA methyltransferase enzymes DNMT3A and DNMT3B are the main proteins responsible for *de novo* methylation; in addition, DNMT3C has recently been reported to be a rodent-specific methyltransferase that is involved in silencing young retrotransposons specifically in the male germline [9], and DNMT3L lacks a functional catalytic domain but serves as an important cofactor for DNMT3A and DNMT3B [10]. Representing a different branch of the DNMT family, DNMT1 is seen as the main maintenance methyltransferase, which after replication is recruited to hemimethylated DNA to ensure faithful propagation of methylation patterns [11]. When the activity of DNMTs is impaired, passive DNA methylation leads to gradual loss of 5meC over several cell divisions. Alternatively, ten-eleven translocation (TET) methylcytosine dioxygenases can initiate active DNA demethylation by catalysing the oxidation of 5meC into 5-hydroxymethylcytosine (5hmC), which subsequently can be processed into unmethylated cytosine by various means [12, 13]. DNMTs and TETs are hence the main regulators of the dynamics of DNA methylation [14, 15].

While promoter methylation has typically been associated with gene silencing, methylation at gene bodies is in contrast positively correlated with transcription, suggesting that the regulation and function of DNA methylation is largely context-dependent [2, 16]. Given the global distribution of DNA methylation along the genome, local context is expected to strongly influence how this mark regulates various biological processes. This context is for a major part defined by another class of epigenetic modifications, in the form of histone marks. DNA is wrapped

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around an octamer of histone proteins (two of each histone H2A, H2B, H3, and H4), of which both the core and tail domains contain a large number of amino acid residues that can be post-translationally modified. The most important and frequently occurring modifications on histones are methylation and acetylation [17]. Depending on their properties, different histone modifications, alone or in combination, could have their own specific effects on transcription, leading to the concept that a potential "histone code" could provide regulatory cues for gene expression. Moreover, numerous studies have investigated the connection between histone marks and DNA methylation, revealing an intriguing interplay [18].

In this review, we will address the crosstalk between DNA methylation and other epigenetic modifications, with a focus on how histone modifications influence recruitment of the different DNMT family members to the DNA. In addition, we will describe several human disease phenotypes that have been associated with aberrant DNMT recruitment, and what these diseases teach us about the molecular mechanisms underlying their specific binding.

Recruitment of DNMT1 to the genome

The first member of the DNA methyltransferase family to be cloned and sequenced in eukaryotes was named DNMT1 [19]. DNMT1 is a multi-domain protein consisting of, from N- to C-terminus, a replication foci targeting sequence (RFTS), a zinc finger CXXC domain, two tandemly connected bromo-associated homology (BAH) domains, and the catalytic methyltransferase (MTase) domain [20] (fig. 1). Furthermore, its non-catalytic N-terminus has been revealed to mediate protein-protein interactions [21]. The preferred substrate for this enzyme was demonstrated to be hemimethylated DNA, suggesting that it could facilitate the semi-conservative inheritance of methyl moieties during DNA replication [22]. Although DNMT1 is the only DNA methyltransferase that harbours this substrate specificity and has therefore been designated as the maintenance DNMT, it in fact also contains considerable *de novo* methylation activity both *in vitro* and *in vivo* [23, 24]. Vice versa, in certain cell types the *de novo* methyltransferases DNMT3A and DNMT3B have been shown to contribute to maintenance methylation as well [25, 26]. While the classical 'division of labour' model of DNMT1 operating in maintenance and the DNMT3s in *de novo* methylation still holds true for certain developmental contexts, it generally is thus an oversimplified, but nevertheless useful framework until all its underlying principles are understood [27, 28].

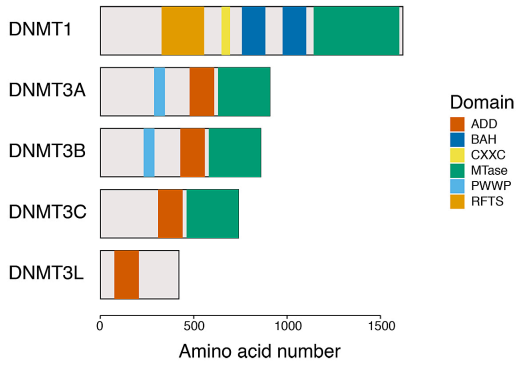
How DNMT1 is specifically recruited to hemimethylated DNA has been extensively studied, revealing a complex mechanism involving several factors. Apart from its inherent preference for hemimethylated cytosine, it was discovered early on that DNMT1 interacts with the DNA clamp proliferating cell nuclear antigen (PCNA), which localises at the replication fork and thereby ensures DNMT1 proximity to newly synthesised DNA [29]. However, the later finding that PCNA is not strictly required for this process indicated that other players must be involved [30]. The most important player was subsequently identified to be the E3 ubiquitin-protein ligase ubiquitin-like with PHD and RING finger domains 1 (UHRF1), knockout of which in mice mimics the *Dnmt1*$^{-/-}$ phenotype [31]. UHRF1 binds to hemimethylated CpG sites via its SET and RING associated (SRA) domain by a base-flipping mechanism, and engages DNMT1 through a direct protein-protein interaction between its own ubiquitin-like (UBL) domain and the RFTS of DNMT1 [32, 33]. In addition, UHRF1 binds to unmodified arginine 2 on histone H3 (H3R2) and to di- and tri-methylated lysine 9 on histone H3 (H3K9me2/3) via its plant homeodomain finger (PHD) and tandem tudor domain (TTD), respectively, thereby contributing to DNMT1 recruitment to different chromatin environments [34–36]. Recent work suggested also a direct interaction between H3K9me3 and the RFTS domain of DNMT1, which would further reinforce the association between this histone mark and maintenance methylation [37].

Adding another layer of epigenetic complexity, UHRF1 catalyses mono-ubiquitinylation of H3 at lysines 14, 18, and 23 [38]. These residues are then bound by the RFTS domain of DNMT1, which is important for both its recruitment as well as its methyltransferase activity [38]. Indeed, binding of the RFTS to ubiquitylated H3 tails releases its auto-inhibitory binding to the MTase domain, leading to enhanced methylation [20]. Recently, an additional ubiquitin-dependent mechanism for DNMT1 recruitment was proposed, in which UHRF1 ubiquitylates PCNA-associated factor 15 (PAF15) on chromatin in a DNA replication-coupled manner [39]. In a mode of action similar to PCNA, PAF15-Ub2 specifically binds to replicating chromatin and forms a complex with DNMT1, thereby contributing to stable maintenance of DNA methylation [39].

Recruitment of the DNMT3 family of proteins to the genome

In contrast to DNMT1, the DNMT3 branch of the DNMT family consists of several closely related members, which in terms of structure represent different variations on the same theme (fig. 1). DNMT3A and DNMT3B are the largest proteins and are built up of a relatively long N-terminal region devoid of any known domains, followed by three chromatin-interacting domains. From N- to C-terminus these are the proline-tryptophan-tryptophan-proline (PWWP) domain, the ATRX-DNMT3-DNMT3L (ADD) domain, and the MTase domain. The rodent-specific DNMT3C enzyme arose through duplication of the *Dnmt3b*

Figure 1: Domain structure of murine DNA methyltransferases. ADD = ATRX-DNMT3-DNMT3L; BAH = bromo-associated homology; MTase = methyltransferase; RFTS = replication foci targeting sequence



gene, but during evolution lost its PWWP domain [40]. DNMT3L likewise lost its PWWP domain, and additionally lacks a functional MTase domain, making it the shortest family member [41]. Although DNMT3L does not possess any inherent DNA methyltransferase ability, it serves as a cofactor for DNMT3A and DNMT3B by directly interacting with these proteins and stimulating their activity, which is particularly important for the establishment and expression of maternally imprinted genes [10, 42, 43].

DNMT3A and DNMT3B are the main enzymes responsible for setting DNA methylation without pre-existing methyl groups being present as a template, more commonly known as *de novo* methylation [44]. Despite their similarity and partial redundancy, neither DNMT3A nor DNMT3B can fully compensate for the loss of the other enzyme, indicating their distinctive functionalities [44]. Although it had been known for a long time that DNMT3A and DNMT3B possess intrinsic affinity for CpG sites, structural insights into this observation were only recently obtained [45–49]. These studies present crystal structures of the catalytic domains of human DNMT3A or DNMT3B in complex with DNMT3L, providing the structural basis for CpG-specific *de novo* DNA methylation.

Furthermore, the observation that DNA methylation is not evenly distributed over the genome suggests that CpG binding by the MTase domain is not the only defining factor for DNMT3 occupancy. Indeed, with the ADD and PWWP domains DNMT3A and DNMT3B possess two chromatin-interacting domains that must be taken into account in this regard. The first indications of the function of the ADD domain came from peptide interaction assays that showed that DNMT3L through its N-terminal region specifically interacts with the tail of histone H3, and that this interaction is abrogated by methylation of H3K4 [50]. That binding to unmethylated H3K4 is a core function of the ADD domain that is conserved across the DNMT3 family was subsequently confirmed by studies with DNMT3A and DNMT3B [51–53]. Moreover, this finding provided an explanation for the previously described anticorrelation between histone H3K4 methylation and DNA methylation [54, 55]. As shown for DNMT3A, binding to H3K4me0 by the ADD domain does not only regulate recruitment, but also stimulates methyltransferase activity through releasing an autoinhibitory interaction with the MTase domain [56].

Yet H3K4me3 is not the only histone modification involved in DNMT3 recruitment. Using peptide arrays, it was shown that the PWWP domain of DNMT3A specifically recognises H3K36me3 [57]. A recent study used chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) and found DNMT3A *in vivo* to be predominantly localised in H3K36me2 and H3K36me3 domains, with preferential enrichment in regions with high H3K36me2 compared to H3K36me3 [58]. This finding was recapitulated *in vitro*, with the DNMT3A-PWWP domain recognising both H3K36me2 and H3K36me3, but exhibiting greater affinity for H3K36me2 [58]. Similar *in vivo* experiments had already uncovered the targeted recruitment of DNMT3B to H3K36me3-decorated bodies of transcribed genes [59]. Since both DNMT3C and DNMT3L lost their PWWP domain, H3K36 methylation-mediated recruitment seems to be specific for DNMT3A and DNMT3B, although these could in turn recruit the other DNMTs through protein-protein interactions [40, 41]. In line with these findings, genome-wide profiling has established a positive correlation between H3K36me2/3 and DNA methylation [58].

Intriguingly, even though H3K36 methylation has been found to negatively correlate with the repressive histone mark H3K27me3, the longer isoform of DNMT3A (DNMT3A1) was discovered to localise to the H3K27me3-positive shores of CGIs [60, 61]. Apart from adding another histone mark to the code defining DNMT3 binding, it also suggests that more isoform-specific binding patterns may exist for DNMTs, since this preferential localisation was not found for the shorter isoform DNMT3A2 [60]. The exact relationship between DNA methylation and H3K27me3 has as yet remained elusive, but genome-wide assessments point to an anticorrelation, and indeed H3K27me3-enriched CGIs have been found to be devoid of DNA methylation [58, 62]. Rather than a direct recruiting function for H3K27me3, this implies recruitment of DNMT3A1 through indirect means, the underlying mechanisms of which must still be further elucidated.

Other mechanisms of DNMT3 recruitment have additionally been described. For example, the histone methyltransferase G9a has been demonstrated to recruit DNMT3A and DNMT3B through a direct interaction with their MTase domains, thereby connecting H3K9 methylation and *de novo* DNA methylation [63]. Similarly, the H3K27 histone methyltransferase EZH2 has been reported to interact with DNMT1 as well as DNMT3A/B and DNMT3L [64, 65], although this recruitment was later shown not to be sufficient for *de novo* DNA methylation and a direct role for Polycomb group proteins in regulating DNA methylation remains enigmatic [66–68]. Further protein-protein interactions between DNMT3s and the histone methyltransferases SUV39H1 and SETDB1 have also been described, suggesting that the link between histone and DNA methylation may be a more general one [69, 70]. However, it remains to be determined to what extent such connections would be causative instead of merely correlative.

Aberrant DNMT recruitment in disease

All in all, recruitment of DNMTs is an intricate process in which the different layers are tightly interwoven. The robustness ensuing from this multi-layered complexity points to an imperative function for proper DNMT localisation in healthy individuals; in line with this, mouse embryos homozygous for knockout of the *Dnmt1* or *Dnmt3b* gene die before birth, while *Dnmt3a*$^{-/-}$ mutant mice become runted and die approximately four weeks after birth [44, 71]. Nevertheless, a few diseases in humans have been described that could be caused by aberrant DNMT recruitment.

Mutations in the RFTS domain of DNMT1 have been associated with two different kinds of neurological disorders. The first one of these is hereditary sensory and autonomic neuropathy type 1 (HSAN1) with dementia and hearing loss, a form of neurodegeneration [72] (table 1). The identified mutations in DNMT1 were shown to result in protein misfolding, decreased enzymatic activity, and impaired heterochromatin binding, leading to the disease phenotype. On the level of the methylome, this translates to

moderate global hypomethylation of cytosines but local hypermethylation at CGIs [72]. The second disease caused by mutations in the DNMT1-RFTS domain is autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCA-DN) [73] (table 1). Interestingly, both conditions are characterised by a late onset, proposing an important role for correct DNA maintenance methylation recruitment in adult neurons.

Regarding the DNMT3 family, aberrant recruitment of mostly DNMT3A has been implicated in disease. Mutations in the *DNMT3A* gene have been widely associated with various haematological malignancies, most frequently with acute myeloid leukaemia (AML), but also including T-cell acute lymphoblastic leukaemia (T-ALL), chronic myelomonocytic leukaemia (CMML), and myelodysplastic syndrome (MDS) [74, 75] (table 1). In addition, *DNMT3A* mutations have been found to cause age-related clonal haematopoiesis (ARCH), which has been associated with not only haematological but also cardiovascular disease [76, 77] (table 1). Although one clear mutational hotspot exists at R882 in the MTase domain, disease-causing mutations have been found to occur over the entire length of the gene, leading to reduced methyltransferase activity, but also decreased or enhanced DNA binding, or impaired ability to multimerise on the DNA. The exact mechanisms by which these mutations drive haematological disease remain to be clarified but are often associated with genome-wide changes in the DNA methylation landscape. Through as yet unknown pathways this influences the cells' ability to switch from self-renewal to differentiation, resulting in various kinds of neoplasms [74].

Furthermore, *DNMT3A* mutations have been linked to abnormal growth phenotypes. In the Tatton-Brown-Rahman syndrome, loss-of-function mutations in *DNMT3A* cause an overgrowth condition paired with mild to moderate intellectual disability [78] (table 1). Interestingly, these mutations are distributed over the PWWP, ADD, and MTase domains, but do not appear in the N-terminal stretch or the regions in between the domains. This suggests that they could interfere with the binding to histone modifications, domain-domain interactions, or both [78]. Reciprocally, recent studies linked gain-of-function mutations in the PWWP domain of DNMT3A to growth retardations [79, 80] (table 1). Whole-exome sequencing of microcephalic dwarfism patients identified two different *de novo* mutations, affecting two amino acids (W330R and D333N) that are part of the aromatic cage that forms around the methylated lysine 36 on histone H3 [82]. Mouse models carrying the murine equivalents of these mutations (W326R and D329A, respectively) displayed growth deficits as demonstrated by reduced brain size and body weight, recapitulating the human disease phenotype [79, 80]. On the molecular level, W326R and D329A were shown in *in vitro* experiments to impair binding of the PWWP domain to H3K36me2/3, suggesting altered binding specificity of DNMT3A to the DNA [57, 79]. Indeed, genome-wide assessment of DNA methylation in two microcephaly patient-derived fibroblast lines identified 1878 common differentially methylated regions (DMRs), the majority of which was hypermethylated relative to healthy controls [79]. Such hypermethylated DMRs were frequently positioned close to key developmental gene loci that were associated with Polycomb-mediated repression in control cells, but showed reduced levels of H3K27me3 in patient fibroblasts [79]. Similar results were obtained by genome-wide DNA methylation profiling in several tissues of adult *Dnmt3a*$^{A/D329A}$ mice [80]. Counterintuitively, increased DNA methylation at these sites leads to de-repression of the associated genes, possibly through displacement of the repressive Polycomb complexes [80]. Interestingly, gain-of-function mutations in the PWWP domain of DNMT3A have also been found in patients with paraganglioma, a type of neuroendocrine tumours [81] (table 1). CRISPR/Cas9-mediated introduction of one of these mutations (K299I) in HeLa cells caused genome-wide alterations in the DNA methylation landscape, suggesting that aberrant DNMT3A recruitment through mutations in the PWWP domain may be a more general disease-causing event [81].

Although DNMT3B is much less implicated in human disease, mutations in the *DNMT3B* gene are well described to cause ICF syndrome, a rare autosomal recessive disease that is characterised by immunodeficiency, centromeric instability, and facial anomalies [83] (table 1). These muta-

Table 1: Diseases associated with aberrant DNMT recruitment.

Disease	Gene	Mutations	Molecular effect	References
HSAN1 (hereditary sensory and autonomic neuropathy type 1 with dementia and hearing loss)	*DNMT1*	Y495C D490E-P491Y	Protein misfolding, decreased enzymatic activity, and impaired heterochromatin binding	[72]
ADCA-DN (autosomal dominant cerebellar ataxia, deafness and narcolepsy)	*DNMT1*	A570V G605A V606F	Not tested, but hypothesised altered DNA binding and/or protein-protein interactions	[73]
Various haematological malignancies (AML, T-ALL, CMML, MDS, ARCH)	*DNMT3A*	Hotspot at R882; other disease-causing mutations over the entire length of the gene	Reduced methyltransferase activity, decreased or enhanced DNA binding, impaired ability to multimerise on the DNA, etc.	[74–77]
Tatton-Brown-Rahman syndrome	*DNMT3A*	Several in the PWWP, ADD, and MTase domains, e.g. I310N M548K R749C	Not tested, but hypothesised altered binding to histone modifications and/or domain-domain interactions	[78]
Growth retardations	*DNMT3A*	W330R	Abrogated H3K36me2/3 interaction	[79]
		D333N		[80]
Paraganglioma	*DNMT3A*	K299I R318W	Not tested, but hypothesised abrogated H3K36me2/3 interaction	[81]
ICF syndrome (immunodeficiency, centromeric instability, and facial anomalies)	*DNMT3B*	Several in both the MTase and PWWP domain, e.g. S282P	Abrogated H3K36me3 interaction	[59, 82–84]

tions have been found not only in the MTase domain, but also in the PWWP domain; one of which (S282P in human, S277P in mouse) was shown by ChIP-seq to abrogate interaction with H3K36me3 [59, 84]. A crystallography study subsequently revealed that this residue stabilises the interaction between methylated H3K36 and a conserved water molecule, building a strong hydrogen bond that is needed for DNMT3B-PWWP substrate recognition [82]. This indicates that H3K36me3-mediated DNMT3B recruitment is essential for healthy cell function.

Future directions

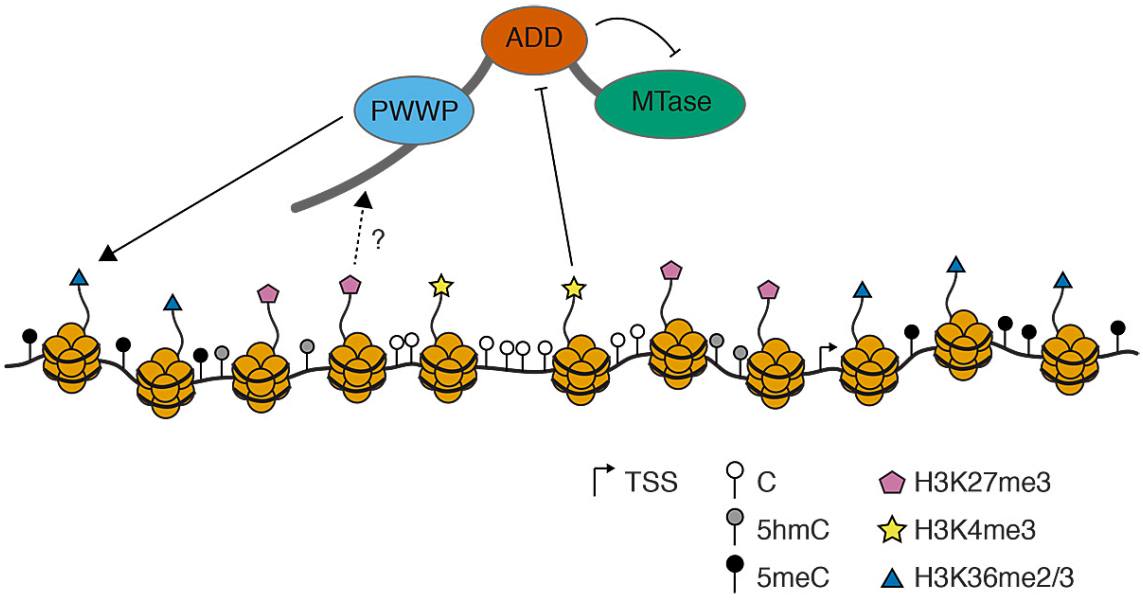
Recruitment of DNMTs to their genomic target sites is a multifaceted process that is dependent on various epigenetic events, which both directly and indirectly contribute to the establishment and maintenance of the DNA methylation landscape. Many such contributing factors have been described, providing important insights into the underlying mechanisms, but at the same time leaving many questions unanswered. Indeed, further research is needed to address these outstanding issues and help construct a complete picture.

For DNMT1, the main histone modifications influencing its recruitment are H3R2, H3Ub, and H3K9me2/3. Not much is known about the gene-regulatory effect of H3R2, methylation of which has been reported to be either associated with euchromatin, or with heterochromatin through antagonising H3K4me3 [85, 86]. The role of H3Ub in transcriptional regulation is likewise not well characterised. In contrast, H3K9 methylation has been studied in more detail, revealing a link to repressed chromatin such as centromeres, transposons, repetitive elements, and imprinted loci [87]. H3K9me2/3 recognition by UHRF1 and subsequent DNMT1 recruitment or by DNMT1 itself thus provides a clear connection between this histone mark and DNA methylation at heterochromatic regions, whose cooperative effect is gene silencing.

The most important players influencing DNMT3 recruitment are H3K4 methylation, H3K27me3, and H3K36me2/3 (fig. 2). H3K4 methylation is a marker for regulatory regions, with H3K4me1 designating enhancers and H3K4me3 promoters [88]. As such, H3K4me3 is enriched at CGIs and anticorrelates with DNA methylation [87]. Interestingly, a subset of CGI promoters is decorated with the activating H3K4me3 and the repressive H3K27me3 histone marks at the same time. This combination has been termed ‘bivalent’, and is believed to ready key developmental genes for rapid activation or repression in a lineage-specific manner [87]. The presence of H3K27me3 by itself is associated with silencing and high levels of CpG methylation throughout most of the genome [62, 88]. At CGIs, however, H3K27me3 and DNA methylation are mutually exclusive, providing an explanation for the unmethylated status of bivalent promoters. Upon global reduction of DNA methylation, H3K27me3 peaks at these sites flatten and spread out, leading to an altered 3D genome through the loss of Polycomb-mediated distal interactions and suggesting that DNA methylation serves to restrict H3K27me3 domains at CGIs [62, 89–91]. The molecular mechanisms behind this redistribution of H3K27me3 in the absence of DNA methylation are hitherto unknown, and also the biological consequences remain an active area of investigation. Additionally, much remains unclear about the role of H3K27me3 in recruiting the DNMT3s, and DNMT3A in particular. To our current knowledge, no domain or region responsible for H3K27me3 recognition has been identified in any of the DNMT3 proteins, arguing against a direct interaction through substrate binding. The fact that the co-occurrence of H3K27me3 and DNA methylation is context-dependent likewise points to more indirect pathways. Future research should therefore investigate which players could constitute the missing link between H3K27me3 and DNMT recruitment.

While H3K4me3 and H3K27me3 are important regulators for DNMT3 localisation at CGIs, H3K36me2/3 is strongly

Figure 2: Histone tail modifications influencing DNMT3A/B recruitment to DNA. The PWWP domain recognises H3K36me2/3 (blue triangles), while the ADD domain is repelled by H3K4me3 (yellow stars). Additionally, the ADD domain auto-inhibits the MTase domain. How H3K27me3 (pink pentagon) influences DNMT3A/B recruitment remains to be elucidated. TSS = transcription start site



enriched across the transcribed bodies of active genes [87]. Both H3K36me2 and H3K36me3 positively correlate with DNA methylation, supporting their function in recruiting DNMT3A and DNMT3B, respectively [58, 59]. Recent studies described mutations in the PWWP domain of DNMT3A that abrogate H3K36 binding, instead recruiting this protein to chromatin that is marked with H3K27me3 in wildtype cells [79, 80]. Previous research had reported that such lowly methylated H3K27me3-marked regions are more likely to contract in the absence of DNMT3A, strengthening the notion that there is crosstalk between DNA methylation and H3K27me3 [92]. Whether this recruitment of mutated DNMT3A is specific or rather a general consequence of increased protein availability is still an open question, the answer to which will teach us more about the dynamics between H3K36me2/3 and H3K27me3 in instructing DNA methylation patterns.

Although recent research has significantly improved our structural understanding of the above-mentioned molecular mechanisms underlying DNMT recruitment, some important information is still missing. Since all structures of DNMT1 that are currently available have been obtained using truncated proteins, the exact domain arrangement of the full-length protein may not have been properly recapitulated [93]. This is especially relevant since interactions between different domains exist that influence the function of the protein, most notably the auto-inhibitory binding of the RFTS domain to the MTase domain [20]. For DNMT3A and DNMT3B it would likewise be useful to compare different stretches of the proteins, which would provide information not only on the function of the domains by themselves or in combination, but also on the naturally occurring isoforms. Additionally, advances in Cryo-EM could help to obtain novel mechanistic insights into DNMT function in the presence of their natural binding substrates of reconstituted nucleosomes carrying distinct modifications.

Furthermore, it would be informative to compare structures of the DNMTs interacting with other family members. Apart from interactions within the DNMT3 branch, DNMT3s are also known to interact with DNMT1 [94], yet if and how these interactions influence conformation and subsequent DNA binding as well as biological function remains to be determined. Such insights could in turn also shed light on the observation that different DNMT3 family members, but also protein isoforms, show differential DNA binding patterns [59, 60]. Besides recruitment of DNMTs to specific chromatin states, recent work suggested structural differences in substrate recognition that could contribute to differential DNMT3A/B activities based on flanking-DNA-sequence around the methylated cytosine [47–49]. Such DNA sequence-mediated differences could strongly contribute to global DNA methylation patterns and provide an additional layer of DNMT regulation, which should be further explored. Some hypotheses on this topic can nonetheless already be formulated based on our current knowledge, for example concerning the DNMT3A/B-DNMT3L interaction. As described, DNMT3L contributes to H3K4me0 binding through its ADD domain, but because of its lacking PWWP domain by definition cannot recognise H3K36 methylation [50]. We can therefore hypothesise that interaction with DNMT3L could strengthen the affinity of DNMT3A or DNMT3B for H3K4me0, while methylation of H3K36 may be less important. It would be interesting to investigate whether the methylation status of H3K4 is indeed more instructive for DNA methylation patterns than that of H3K36, and if this is correlated to the expression of DNMT3L.

All in all, although various pathways influencing DNMT recruitment to DNA have been charted, how exactly they should be navigated remains to be determined. Dissecting these mechanisms will aid our understanding of the crosstalk between different histone modifications and DNA methylation, and how this together influences the genome-wide epigenetic landscape. Yet this is still only part of the whole story, since factors like protein-protein interactions, post-translational modifications, and RNAs have also been described to be involved in recruiting DNMTs to their genomic target loci [93]. In addition to mechanisms that recruit DNMTs to the genome, allosteric regulation [93] and structural constrains that influence catalytic activity based on DNA sequence context [47, 95, 96] are expected to contribute to DNA methylation regulation. Continued efforts will be directed towards solving this intriguing puzzle.

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