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Regulation and function of mammalian DNA methylation patterns: a genomic perspective

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Abstract

Mammalian DNA methyltransferases (DNMTs) establish and maintain genomic DNA methylation patterns that are required for proper epigenetic regulation of gene expression and maintenance of genome stability during normal development. Aberrant DNA methylation patterns are implicated in a variety of pathological conditions including cancer and neurological disorders. Rapid advances in genomic technologies have allowed the generation of high resolution whole-genome views of DNA methylation and DNA methyltransferase occupancy in pluripotent stem cells and differentiated somatic cells. Furthermore, recent identification of oxidation derivatives of cytosine methylation in mammalian DNA raises the possibility that DNA methylation patterns are more dynamic than previously anticipated. Here, we review the recent progress in our understanding of the genomic function and regulatory mechanisms of mammalian DNA methylation.

Keywords: *DNA methylation; DNA methyltransferases; Tet DNA hydroxylases; Polycomb repression; promoter and non-promoter DNA methylation; epigenomics; methylome*

INTRODUCTION

Epigenetics in its modern terms refers to molecular mechanisms that determine the inheritable cellular phenotype without altering the genotype [1, 2]. Epigenetic mechanisms constitute a wide range of molecular processes, such as histone modification, DNA methylation, nucleosome remodeling and non-coding RNAs [3, 4]. Cell-type specific epigenetic modifications may persist through many cell divisions, thereby allowing cells with identical genomes to stably display their unique gene expression profiles and cellular phenotypes [5].

Methylation of the 5′-position of cytosine (5-methylcytosine or 5mC) is a conserved epigenetic mechanism in plants and animals. DNA cytosine methylation contributes to diverse biological processes, such as cellular differentiation, retrotransposon silencing, genomic imprinting and X chromosome inactivation [2]. In mammals, DNA cytosine methylation predominantly occurs at symmetrical CpG dinucleotides. Three catalytically active enzymes, Dnmt1, Dnmt3a and Dnmt3b, are required for establishment and maintenance of DNA methylation profiles [6]. New DNA methylation patterns are initially established by *de novo* DNA methyltransferases, Dnmt3a and Dnmt3b [7, 8]. Dnmt3l, a close homolog of Dnmt3a and Dnm3b, lacks the catalytic domain but may form a complex with Dnmt3a

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and/or Dnmt3b and stimulate their enzymatic activities [9]. Maintenance of existing pattern of DNA methylation during cell division is primarily mediated by Dnmt1, which is recruited by Uhrf1 to DNA replication foci and preferentially methylates hemi-methylated CpG sites [10–13]. All three DNA methyltransferases are essential for normal development as genetic deletion of Dnmt1 or Dnmt3b results in embryonic lethality and homozygous Dnmt3a knockout mice die 1 month after birth [7, 14]. Furthermore, mutations in *DNMT3B* gene cause immunodeficiency–centromeric instability–facial anomalies (ICF) syndrome. Mutations of *MECP2* gene, which encodes a methyl-CpG-binding protein, lead to a debilitating neurological disorder termed Rett syndrome [7, 15, 16]. DNA methylation patterns are also known to be frequently altered in imprinting disorders and cancer [17, 18].

DNA methylation patterns are relatively stable in tissue-specific somatic cells. So DNA methylation has long been considered to be a major epigenetic mechanism for stable gene silencing. In support of this notion, DNA methylation at the gene promoter can either block the binding of transcriptional factors or facilitate the recruitment of methyl-CpG-binding domains (MBDs)-associated co-repressor complexes [2]. However, genome-wide erasure of DNA methylation and subsequent re-methylation is observed in two specific developmental stages: the zygotes/pre-implantation embryos [19, 20] and developing primordial germ cells (PGCs) [21, 22]. The resetting of DNA methylation patterns is believed to be critical to establish a pluripotent state in pre-implantation embryos and setup parental-origin-specific imprints in developing PGCs [21, 23]. Such large-scale dynamic changes of DNA methylation suggest the presence of enzymes that can efficiently erase or modify cytosine methylation patterns [24]. Indeed, ten-eleven translocation (Tet) proteins have recently been identified in human and mouse to be capable of converting 5mC into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) through iterative oxidation reactions [25–31]. In addition, recent genome-wide methylome analyses in pluripotent stem cells and differentiated cells have revealed a more complex, context-dependent role for DNA methylation in transcriptional regulation. These genome-wide DNA methylation studies indicate that not only is promoter methylation more dynamic than previously thought, but actively transcribed genes tend to be associated with high levels of gene body DNA methylation [32–34]. Moreover, genome-wide mapping and *in vivo* functional analyses of the *de novo* DNMTs suggest that DNA methylation may play a role in both gene repression and activation [35–38].

Here, we review how recent advances in determining mammalian DNA methylation and DNMT occupancy on a whole-genome scale have provided new insights into the gene regulatory function of DNA methylation. We begin by reviewing the important methodological development towards genome-wide mapping of DNA methylation. We then summarize the recent genome-wide studies on the distribution and functions of DNA methylation and DNMTs at both promoter and non-promoter regions, and conclude by highlighting several remaining outstanding questions in DNA methylation regulation.

TECHNOLOGIES FOR GENOMIC ANALYSIS OF METHYLOME AND DNMT DISTRIBUTION

While genetic, biochemical and structural studies have provided insights into the functions of DNA methyltranferases, it has been largely unclear whether the conclusions drawn from locus-specific analysis can be generally applied to other regions in the genome. To understand the biological significance of DNA methylation, it is essential to determine the genome-wide distribution of DNA methylation and related epigenetic marks (e.g. histone methylation). Recent technological advances have allowed generation of methylome maps at single-nucleotide resolution in pluripotent stem cells and differentiated cells [32, 39–43], which have revolutionized our understanding of the functions of this critical epigenetic mark during development [33].

Classical technique used for detection of 5mC is based on the sensitivity of restriction enzymes to CpG methylation within their cleavage sites [44]. While methylation-sensitive restriction enzymes can be combined with microarrays or high-throughput sequencing (e.g. HELP assay, HpaII tiny fragment enrichment by ligation-mediated PCR) [45], such method is inherently constrained by the position of recognition sites and thereby only provides a limited coverage of 5mC detection. More recently, fractionation with the methylation-specific enzyme *Mcr*BC [46] and optimized custom

tiling arrays have been used to improve the performance of restriction enzyme-based genome-scale DNA methylation analysis (e.g. CHARM assay, comprehensive high-throughput arrays for relative methylation) [47, 48]. In addition, unmethylated CpG sites, especially in cluster, can be readily identified by high-throughput sequencing of size-selected fragments derived from DNA digestions with a cocktail of methyl-sensitive restriction enzymes (e.g. MRE-seq) [49].

To circumvent limitations associated with restriction enzymes, affinity purification approaches have been developed to enrich methylated or unmethylated DNA fragments. In one method termed methylated DNA immunoprecipitation assay (MeDIP or mDIP), a monoclonal antibody specifically recognizing 5mC is utilized to purify denatured 5mC-containing genomic DNA [50, 51]. Alternatively, methylated native DNA fragments can also be isolated by using immobilized MBDs that are derived from MBD containing proteins such as MeCP2 and MBD1-3 [52, 53]. In contrast, the Cys-X-X-Cys (CXXC) domain, which has high affinity for unmethylated CpG sites, can be utilized to enrich specifically for unmethylated DNA fragments [54]. Combined with whole-genome tiling microarrays, MeDIP-chip and/or MBD-chip assays have generated the first genome-wide methylome map in *Arabidopsis thaliana* [55, 56]. Comparative analyses of these enrichment methods (MeDIP-seq versus MBD-seq) indicate that they may generate comparable results when coupled with high-throughput sequencing [57, 58]. Notably, MeDIP-seq may potentially be used to detect DNA methylation in a strand-specific manner and in non-CpG context, whereas MBD-seq is limited in these applications as MBD domains only recognize symmetrically methylated CpG sites in native/non-denatured DNA duplex. Similar to affinity enrichment methods for detecting DNA methylation, Chromatin immunoprecipitation followed by genomic tiling microarray hybridization (ChIP-chip) or massively parallel sequencing (ChIP-seq) has merged as a powerful technique for genome-wide analysis of DNA methyltransferase or MBD containing proteins [35, 59, 60].

While MeDIP/MBD-seq can provide comprehensive methylome coverage at relatively low cost, these approaches are generally of moderate resolution (200–500 bp) [57, 58]. Moreover, one caveat with affinity enrichment based methods is that the immunoprecipitation efficiency is partially dependent on CpG density such that methylated CpG sites within CpG-poor sequences may not be reliably detected [51]. In contrast, bisulphite conversion followed by Sanger sequencing can determine methylation states of CpG sites at single base-pair resolution [61]. This method depends on the fact that prolonged incubation with sodium bisulphite only converts cytosines, but not 5-methylcytosines, to uracil. Upon PCR amplification, 5mC and C will be interpreted as C and T, respectively. However, large-scale Sanger sequencing of bisulphite converted DNA is time- and resource-intensive [62]. More recently, rapid development of next-generation sequencing technologies has allowed direct shotgun sequencing of bisulphite treated genomic DNA (BS-seq or MethylC-seq) to generate single-nucleotide resolution map of methylomes in plant [63, 64], mouse [34, 43, 63] and human [32, 39–41, 65–67]. Targeted sequencing strategies using restriction enzymes (RRBS, reduced representation bisulphite sequencing) or sequence-specific padlock probes have also been reported [34, 42]. Furthermore, several platforms with higher throughput of sample processing, such as 454 sequencing-based Pyrosequencing, mass spectrometry (MS)-based Sequenom EpiTYPER assays and microarray hybridization-based Illumina Infinium [44], have been developed to facilitate the validation of results from genome-wide DNA methylation analysis. However, it is worth noting that bisulphite conversion or restriction enzyme-based DNA methylation detection methods can not readily discriminate 5mC from recently discovered 5mC oxidation derivatives such as 5hmC [68, 69].

REGULATORY MECHANISMS OF DNA METHYLATION PATTERNS AT MAMMALIAN GENE PROMOTERS

Unlike organisms with little or no DNA methylation (e.g. *Caenorhabditis elegans* and *Saccharomyces cereviceae*), CpG dinucleotides in mammalian genomes are unevenly distributed and clustered to form discrete CpG-rich regions (CpG-islands or CGIs), which are believed to be associated with ~70% of human gene promoters [70]. While most CpG sites in the mammalian genome are methylated, CGIs are generally unmethylated [70]. Even during development, only a small number of CGIs, primarily those in intragenic regions, become methylated [70]. Thus,

the bimodal DNA methylation pattern in the mammalian genome is relatively stable and likely to be important for genomic regulation in normal cells. Interestingly, cancers cells are frequently associated with global DNA hypomethylation and tumor suppressor gene promoter hypermethylation [18]. Currently, little is known about the developmental origin of this bimodal DNA methylation pattern and the molecular mechanisms that govern its establishment. One possibility is that the global DNA methylation pattern is established by the inherent binding affinity of *de novo* DNA methyltransferases to chromatin. In support of this hypothesis, both Dnmt3a and Dnmt3b, but not Dnmt1, are found to be stably associated with nucleosomes with high levels of DNA methylation [71]. In contrast, targeting of DNMTs to key regulatory regions such as gene promoters may be positively or negatively regulated by specific mechanisms, such as chromatin states and transcription factor binding [72, 73]. Integrated genome-wide analyses of human and mouse cells have shown that the majority of CGI-containing gene promoters are marked by the transcriptionally permissive/active histone mark, di-/tri-methylation of lysine 4 on histone H3 (H3K4me2/3) [74, 75], and are associated with a DNA hypomethylated state [34, 51]. These observations led to the proposal that active histone marks may play a critical role in regulating DNMT targeting at gene promoters. Mechanistic insights into this proposed regulatory process came from biochemical and structural analyses of the Dnmt3l/Dnmt3a complex in mouse embryonic stem (ES) cells [73, 76]. Dnmt3l, the catalytically inactive member of Dnmt3 family, is highly expressed in mouse ES cells and germ cells; together with Dnmt3a, Dnmt3l is critically involved in establishing genomic imprints [9, 77, 78]. In mouse ES cells, Dnmt3l not only interacts with Dnmt3a2 (an N-terminus truncated shorter isoform of Dnmt3a that is highly expressed in mouse ES cells) and Dnmt3b, but also with core histones [73]. Specifically, the ADD (ATRX–Dnmt3–Dnmt3l) domain of Dnmt3l is found to selectively interact with the N-terminus tail of histone H3 when H3K4 is unmethylated [73, 76], thereby targeting catalytically active DNMTs to nucleosomes free of H3K4 methylation. In agreement with this, genetic deletion of KDM1B, a H3K4 demethylase that is highly expressed in growing oocytes where genomic imprints are established, resulted in significant increase in H3K4 methylation and a failure to set up the DNA methylation marks at several imprinted loci [79]. Further analyses have showed that Dnmt3a and Dnmt3b isoforms expressed in somatic cells are also capable of discriminating the methylation state of H3K4 via their ADD domains [80, 81]. A recent genome-wide analysis of Dnmt3a occupancy in postnatal neural stem cells suggests that Dnmt3a is indeed excluded from CpG-rich, H3K4me3-marked gene promoters, but frequently binds to genomic regions flanking H3K4me3-marked nucleosomes or CpG-poor/inactive gene promoters [35] (Figure 1). These results thus lead to a model in which H3K4 methylation may inhibit *de novo* DNA methylation at gene promoters or imprinting loci by opposing the recruitment of *de novo* DNA methyltransferases.

H3K4me3 enriched at CpG-rich gene promoters are catalysed by MLL and SET1 family of H3K4 methyltranferases. Both MLL and Cfp1 (an interacting partner of SET1) proteins contain a CXXC domain [82], which has a high affinity for clustered unmethylated CpG sites, suggesting a role of CXXC domain in recruiting H3K4 methyltransferases to CGIs and regulating DNA methylation [70, 83]. Interestingly, the CXXC domain is also found in TET1, the founding member of a family of Fe(II)- and 2-oxoglutarate (2-OG)-dependent dioxygenases that is capable of converting 5mC to 5hmC, 5fC and 5caC, through iterative oxidation reactions [25, 28–31]. These findings raise the possibility that TET family of DNA hydroxylases may contribute to the hypomethylated status of CGIs. Indeed, recent genome-wide studies of Tet1 occupancy in mouse ES cells suggest that Tet1 is enriched at CpG-rich gene promoters and intragenic sequences [84–86]. In support of a role of Tet proteins in modulating DNA methylation patterns at their binding sites, Tet1 bound CGIs are hypomethylated and depletion of Tet1 in mouse ES cells results a aberrantly increase in DNA methylation at many Tet1 binding sites [85–87]. It is currently unclear how Tet proteins promote DNA demethylation at CpG-rich gene promoters. One possibility is that Tet proteins can directly remove sporadic *de novo* DNA methylation at CGIs by converting 5mC to its oxidation derivatives and initiating an active or passive process of demethylation [85]. In support of this model, genome-wide mapping of 5hmC in mouse ES cells indicated that this oxidized form of 5mC is enriched at many gene promoters with high/moderate density of CpG sites [85–89]. Thus, Tet

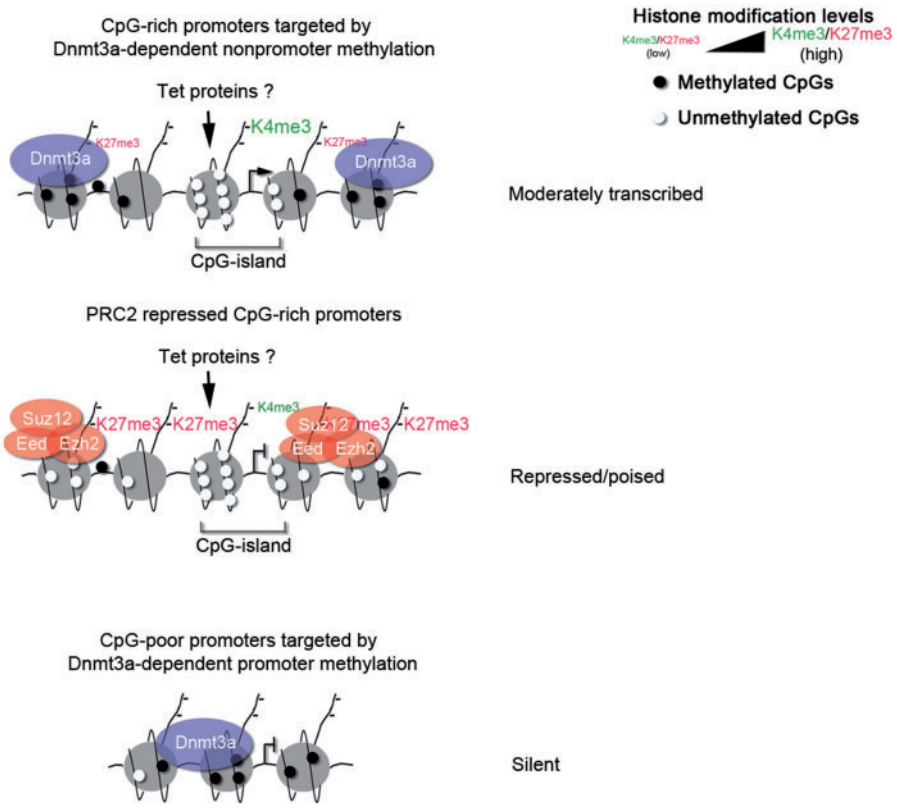

Figure 1: A proposed model of Dnmt3a-dependent promoter and non-promoter DNA methylation in repressing and facilitating gene expression,respectively, in postnatal neural stem cells. In postnatal neural stem cells, Dnmt3a-mediated non-promoter DNA methylation is found at a cohort of actively transcribed genes with CpG-rich promoters. In contrast, a subset of CpG-rich promoters is silenced by PRC2-mediated repression and is associated with high levels of H3K27me3. In addition, a subset of transcriptionally inactive, CpG-poor promoters is targeted by Dnmt3a-dependent promoter methylation. Note that Tet family of DNA hydroxylases may contribute to maintain the DNA hypomethylated state at CpG-islands.

protein-mediated 5mC oxidation may act in concert with H3K4me3-dependent repulsion of DNMT binding to ensure an unmethylated state at CpG-rich, transcriptionally active promoters.

In addition to CXXC domain-containing proteins (e.g. MLL/SET1 H3K4 methyltransferases and Tet DNA hydroxylases), other mechanisms may contribute to regulation of DNA methylation patterns at transcriptionally active gene promoters. For instance, recent genome-wide studies of H2A.Z, a histone variant that frequently resides at transcriptional start sites of active promoters and is implicated in transcriptional initiation, have indicated that H2A.Z deposition inversely correlates with the presence of DNA methylation, and suggested a role for H2A.Z in antagonizing DNMT binding [90, 91]. Furthermore, it has been proposed that local DNA sequences within gene promoters, particularly transcription factor binding sites, may act as intrinsic determinants of proximal promoter DNA methylation patterns [72]. Recent whole-genome BS-seq analyses in mouse ES cells and neuronal progenitors have provided strong evidence that transcription factor (e.g. CTCF, REST) binding may be critically involved in determining the methylation states at their binding sites [43]. However, it is currently unclear how sequence-specific transcription factor binding modulates DNA methylation. It is possible that both replication-dependent dilution and Tet protein-initiated active DNA demethylation may facilitate this process.

While majority of CpG-rich gene promoters are unmethylated regardless of their transcriptional status, many CpG-poor gene promoters are methylated [51]. It has been proposed that interplay between histone modification enzymes and DNMTs

may play a critical role in directing DNA methylation to inactive gene promoters [92]. The H3K9me2 methyltranferase G9a has been shown to recruit DNMTs to methylate a subset of pluripotency gene promoters [93]. Subsequent analysis suggests that G9a may direct DNA methylation to target promoters independent of its methyltransferase activity [94, 95], suggesting that H3K9 methylation per se is not required for recruiting DNA methylation. Instead, G9a can potentially recruit Dnmt3a and Dnmt3b via its ankyrin domain [96]. Similarly, a related H3K9 methyltransferase, SETDB1 (also known as ESET) may recruit DNMT3A to repress gene promoters in cancer cells [97]. PRMT5-mediated H4R3 methylation marks (H4R3me2) may also serve as a binding site for the ADD domain of DNMT3A [98]. Interestingly, during ES cell differentiation or in cancer cells, gene promoters associated with the repressive histone mark H3K27me3 have been found to have a strong tendency to be DNA methylated [99–102]. It suggests a direct crosstalk between components of Polycomb repression complex 2 (PRC2), which catalyses the H3K27 methylation [103], and DNMTs in cancers [104]. However, integrated genome-wide analysis of DNA methylation and H3K27me3 suggest that these two epigenetic marks are generally associated with different sets of gene promoters in both mouse ES cells and in cancer cells [105, 106], suggesting that these two epigenetic marking systems are largely not co-localized. Thus, future studies are needed to characterize in detail the mechanistic relationship between PRC2/H3K27me3 and DNA methylation during development and in cancer cells. Together, these studies have clearly established a link between histone modification and DNA methylation. But many questions remain regarding the specific contributions of these histone modification enzymes to the establishment of DNA methylation patterns during development. Integrated genome-wide analyses of histone modification and DNA methylation patterns may shed new light on these issues.

REGULATORY MECHANISMS AND FUNCTIONS OF EUCHROMATIC NON-PROMOTER DNA METHYLATION PATTERNS

Recent whole-genome methylome studies of human cells indicate that most methylated CpG sites are located outside gene promoters [32, 47, 107]. While it is well established that DNA hypermethylation at promoters and repetitive sequences is involved in stable silencing, it is largely unclear how euchromatic non-promoter DNA methylation contributes to regulation of gene expression. Interestingly, a large-scale bisulphite sequencing analysis of multiple human tissues indicates that most differentially methylated regions are found in intragenic and intergenic regions [62]. Using CHARM assays to interrogate whole-genome methylation patterns of a panel of normal mouse tissues and human colon cancer samples, recent studies indicate that tissue-specific DNA methylation is frequently localized in regions flanking CGIs (or CpG-island shores), but not within CGI-containing promoters [47]. In agreement with the proposal that CpG-island shore DNA methylation highly correlates with and potentially regulates tissue-specific gene expression profiles, such non-promoter DNA methylation has been implicated in regulating hematopoietic lineage commitment and epigenetic memory during somatic cell reprogramming [108, 109].

Single-nucleotide resolution methylome mapping by whole-genome shotgun bisulphite sequencing (BS-seq or MethylC-seq) have further revealed unexpected features and potential functions of non-promoter DNA methylation in mammalian genome. Comprehensive mapping of DNA methylation at single-nucleotide resolution in human ES cells and fibroblasts indicates that extensive DNA methylation in non-CpG context (CpH, H = A, C and T) is present in pluripotent stem cells [32]. While nearly one-quarter of all methylcytosine identified in human ES cells are in non-CpG context [32], the functional significance of non-CpG methylation is largely unknown. Intriguingly, non-CpG methylation in human ES cells is significantly enriched in gene bodies of actively transcribed genes and exhibits a preference for the antisense strand of intragenic regions. The fact that a large fraction of non-CpG methylation is asymmetrical in the genome raises the question as to how the non-CpG methylation is established and maintained. The non-CpG methylation shows a sequence preference for CpA and a periodicity of 8–10 bases [32], both of which are consistent with the possibility that mammalian non-CpG methylation is also mediated by *de novo* DNA methyltransferases DNMT3A/B [110].

In addition to non-CpG methylation, whole-genome methylome mapping studies have identified many large genomic domains that are differentially methylated between human ES cells and somatic cells (on both autosomes and X chromosome) [32, 39]. These regions, which are termed as partially methylated domains (PMDs), are characterized by hypomethylation and are preferentially found in somatic cell types [32, 111]. Interestingly, genes within PMDs are generally associated with lower levels in gene expression, decreased intragenic DNA methylation and H3K36me3, as well as reduced levels of promoter H3K4me3; by constrast, PMDs are frequently associated with higher levels of repressive histone modifications (e.g. PRC2-mediated H3K27me3) [32, 66]. Thus, non-promoter DNA methylation may be required for facilitating transcription of genes within PMDs. In support of this possibility, a recent genome-wide study of Dnmt3a in postnatal neural stem cells indicate that Dnmt3a-dependent non-promoter methylation may promote expression of a cohort of actively transcribed genes including neurogenic genes by antagonizing PRC2-mediated repression and reducing H3K27me3 levels [35] (Figure 1). The antagonistic relationship between DNA methylation and H3K27me3 has also been reported in human cancer cell lines or on the regulatory region of an imprinting locus [112, 113]. Consistently, *in vitro* chromatin recruitment assays demonstrate that DNA methylation in a nucleosomal context is capable of repelling components of PRC2 [35, 114].

Integrated genome-wide studies of methylated (MeDIP-seq) and unmethylated regions (MRE-seq) have also implicated intragenic DNA methylation in the regulation of alternative promoter usage [49]. Furthermore, gene body methylation has shown a strong positive correlation with the presence of H3K36me3 [42, 115], a histone mark specifically associated with actively transcribed intragenic regions. Interestingly, both H3K36me3 and intragenic DNA methylation are found to exhibit a relative enrichment within exons over introns [115–117], raising the possibility that they are involved in regulating alternative splicing. Indeed, a recent study has shown a functional link between intragenic DNA methylation and CCCTC-binding factor (CTCF)-mediated splicing regulation [118]. Since CTCF plays essential roles in chromatin looping/organization and CTCF binding to its target sites is sensitive to DNA methylation status, DNA methylation may be involved in regulation of genomic architecture. Furthermore, both CpG and non-CpG methylation may also contribute to regulation of distal regulatory elements (e.g. enhancers), which are characterized by deoxyribonuclease 1(DNase1) sensitivity, nucleosome depletion, and the presence of specific histone marks such as H3K4me1 and H3K27 acetylation (H3K27ac) [32, 41, 119] . Recent studies of 5mC oxidation derivatives further suggest that 5hmC may be enriched at active enhancers [85, 87–89, 120, 121], suggesting a role of Tet-mediated 5mC oxidation in regulating enhancer functions.

CONCLUDING REMARKS AND PERSPECTIVES

In summary, increasing application of genomic technology in studying mammalian methylomes has revolutionized our knowledge of how DNA methylation patterns are established and regulated. The comprehensive and high-resolution map of DNA methylation patterns in various cell types and tissues have also provided new insights into gene regulatory functions of both promoter and non-promoter DNA methylation.

Despite these significant progresses, important questions still remain regarding the dynamic regulation of DNA methylation and demethylation as well as the potential involvement of non-coding RNAs in targeting *de novo* DNMTs to specific sequences in the mammalian genome. First, it is now accepted that euchromatic DNA methylation can be dynamically regulated during lineage commitment and in case of stimuli-induced gene regulation in adult organisms (e.g. neuronal plasticity in postnatal brains) [24, 122–125]. Recent demonstration that Tet protein-mediated 5mC oxidation may initiate both active and passive DNA demethylation raises the possibility that Tet hydroxylases and *de novo* DNMTs may cooperate in regulating the dynamic changes in DNA methylation on developmentally regulated genes [24]. The fact that 5hmC are enriched in mouse ES cells and brain tissues [126, 127] suggests that DNA methylation patterns may not be irreversibly 'locked' in tissue-specific cells and can potentially be regulated in response to differentiation signals and external stimuli. Additional integrated analysis of 5mC and its oxidation derivatives during cellular differentiation and in terminally differentiated cells (e.g. neurons) may provide new insights into the function of 5mC oxidation in gene regulation and their biological significance.

Second, it is currently unclear how *de novo* DNMTs and Dnmt3l are guided to target specific sequences in the genome. Recent studies of mouse male germ cells deficient in Piwi subfamily of Argonaute proteins suggest that the Piwi-interacting RNAs (piRNAs), a class of small non-coding RNAs, may play a role in targeting DNMTs to transposon sequences [128–130]. Additional studies are needed to elucidate the potential molecular mechanism of RNA-based recruitment of DNMTs and explore the possibility that it may act as a more general mechanism that also operates in somatic cells.

Besides the essential roles of DNA methylation in developmental regulation, the link between alterations in DNA methylation patterns and cancers is now well established. Recent identification of mutations in DNMT3A and TET2 in leukemia has further highlighted the importance of enzymes that establish and modify DNA methylation patterns in tumorigenesis [131–133]. Large-scale cancer methylome studies [65–67] will undoubtedly advance our knowledge about the role of this critical epigenetic modification in human health and inform development of effective therapeutic strategies targeting aberrant DNA methylation.

Key Points

- Mammalian DNA methylation plays essential roles in normal development and gene regulation.
- DNA methylation patterns are established and maintained by DNA methyltransferases (DNMTs).
- Aberrant DNA methylation is implicated in various pathologies such as cancer and neurological disorders.
- Recent genome-wide studies of DNA methylation patterns (methylomes) and DNMT occupancy have provided exciting new insights into the regulatory mechanisms and genomic function of both promoter and non-promoter (gene body and intergenic) DNA methylation.

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