Regulation of mammalian DNA methyltransferases: a route to new mechanisms

Hélène Denis*, Matladi N. Ndlovu* & François Fuks⁺

Laboratory of Cancer Epigenetics, Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium

DNA methyltransferases (DNMTs) establish and maintain DNA methylation patterns at specific regions of the genome, thereby contributing to gene regulation. It is becoming evident that an intricate web of pathways target DNMTs to these genomic regions. Here, we review the understanding of these regulatory mechanisms and provide an overview of the new findings, emphasizing the emerging scenario in which several levels of regulation are coordinated to control DNMTs. The mechanisms involved include the dynamic interplay between interdependent post-translational modifications that regulate DNMTs, post-transcriptional regulation by miRNAs and the emerging role of non-coding RNA in targeting mammalian DNMTs. The analysis of these mechanisms is imperative to the understanding of the role of DNA methylation in regulating gene expression during development and in disease.

Keywords: DNA methyltransferases; DNA methylation; PTM; non-coding RNAs

EMBO reports (2011) 12, 647–656. doi:10.1038/embor.2011.110

See Glossary for abbreviations used in this article.

Introduction

DNA methylation is a key mechanism of epigenetic regulation in eukaryotes. In mammals, DNA methyltransferases (DNMTs) establish and maintain methylation of the fifth carbon of cytosine residues in DNA within CG dinucleotides. Genomic methylation patterns are established in at least two developmental periods—in germ cells and preimplantation embryos—generating cells with broad developmental potential. Maintenance mechanisms then ensure the inheritance of proper DNA methylation patterns in somatic differentiated cells (Reik *et al*, 2001). In addition, mammalian DNA methylation has a crucial role in maintaining pluripotency, X-chromosome inactivation and genomic imprinting (Bird, 2002). DNA methylation also protects genome integrity by silencing transposable elements to ensure chromosome stability (Goll & Bestor, 2005; Howard *et al*, 2008). Aberrant DNA methylation is the best-characterized epigenetic hallmark for several pathologies, including cancer (Robertson, 2005). Cancer cells are characterized by the loss of both global and

gene-specific DNA methylation, as well as by hypermethylation of specific promoters (Jones & Baylin, 2007; Portela & Esteller, 2010). Furthermore, dysregulated expression of DNMTs has been reported for various human cancers including hepatomas, as well as prostate, colorectal and breast cancers (Miremadi *et al*, 2007), and defects in some DNMTs lead to mouse embryonic lethality (Li *et al*, 1992).

There are several methods by which to map the genome-wide distribution of methylated DNA residues, such that entire ‘methylomes’ can be characterized at single base-pair resolution. These studies have given surprising insights into the dynamics of DNA methylation, including the presence of extensive 5meC in a non-CG sequence context (Laurent *et al*, 2010; Lister *et al*, 2009), and confirmed that gene-body methylation in actively transcribed genes is a consistent phenomenon throughout the human genome (Ball *et al*, 2009; Laurent *et al*, 2010). These data and others have raised intriguing questions about how the DNA methylation machinery is directed to specific sequences to establish complex DNA methylation patterns. In this review, we begin by considering how the establishment and maintenance of DNA methylation is controlled in mammals. We then examine key aspects of the mechanisms that target mammalian DNMTs, including the involvement of non-coding-RNAs (ncRNAs) in mediating CG methylation in mammals. Furthermore, we discuss the recently identified post-transcriptional regulation of DNMTs by microRNAs (miRNAs) and finally provide an update on the regulation of DNMT activity by post-translational modifications.

Targeting DNMTs: an overview

Structural insights

In mammalian genomes, DNMTs are the only enzymes that have been shown to mediate the transfer of a methyl group from S-adenosylmethionine (SAM) to cytosine (Goll & Bestor, 2005). There are three enzymatically active mammalian DNMTs—DNMT1, DNMT3A and DNMT3B—and one related regulatory protein, DNMT3L, which lacks catalytic activity (Fig 1A). DNMTs are essential for the establishment of cytosine methylation patterns, as well as for their maintenance throughout cell replication (Goll & Bestor, 2005). DNMT1 is primarily a maintenance methyltransferase that preserves methylation patterns during cell division. It localizes to DNA replication foci during S phase, at which it preferentially methylates hemimethylated CG dinucleotides through its interaction with UHRF1 (Avvakumov *et al*, 2008; Sharif *et al*, 2007). There is also evidence of DNMT1 *de novo* activity in human cancer cells (Jair *et al*, 2006) and in maintaining genome stability (Chen *et al*, 1998).

Laboratory of Cancer Epigenetics, Faculty of Medicine, Université Libre de Bruxelles, 808 Route de Lennik, 1070 Brussels, Belgium

*These authors contributed equally to this work.

⁺Corresponding author: Tel: +32 2 555 62 45; Fax: +32 2 555 62 57;

E-mail: ffuks@ulb.ac.be

Received 24 February 2011; accepted 24 May 2011; published online 10 June 2011

Glossary

CDKL5	cyclin-dependent kinase-like 5
CK1	casein kinase 1
HDAC	histone deacetylase
HuR	Hu-antigen R
KDM	lysine demethylase
KMT	lysine methyltransferase
mRNA	messenger RNA
PCNA	proliferating cell nuclear antigen
PRMT5	protein arginine methyltransferase 5
PKC	protein kinase C
SAM	S-adenosylmethionine
UHRF1	ubiquitin-like, containing PHD and RING finger domains 1
UTR	untranslated region

Although DNMT1 was the first-identified eukaryotic methyltransferase (Bestor *et al*, 1988), the crystal structures of mouse DNMT1 (residues 650–1602) and human DNMT1 (residues 646–1600) have only been solved recently (Song *et al*, 2011). This report revealed that active DNMT1—in complex with SAM and duplex DNA containing unmethylated CG sites—undergoes conformational changes. The CXXC domain of DNMT1 binds specifically to unmethylated CG sites and positions the CXXC–BAH1 linker between the unmethylated DNA and the active site of DNMT1, thereby preventing *de novo* DNA methylation (Fig 1A). As such, the authors propose that auto-inhibition of *de novo* methylation is essential to increase the efficiency of maintenance methylation by DNMT1. The crystal structure of DNMT1 bound to hemimethylated DNA needs to be solved in order to understand DNMT1 substrate recognition. One of the key questions is what defines the intrinsic preferences of DNMTs for certain DNA sequences, whether these sequences encompass hemimethylated CG, unmethylated CG or the recently affirmed non-CG substrates.

In this context, DNMT1 has been shown to be dependent on the SRA domain of UHRF1 for its recruitment to hemimethylated CG sites (Sharif *et al*, 2007). For the DNMT3 enzymes, which are responsible for *de novo* methylation during embryonic development, several models have been proposed to explain the way in which they are targeted to genomic regions. These models mainly involve the inherent properties of DNMT3 enzymes and the influence of their interacting partners, which operate either separately or synergistically. It has been suggested that the intrinsic sequence preferences of DNMT3 enzymes are important for global *de novo* methylation, whereas crosstalk with other factors seems to be more important for locus-specific DNA methylation (Jurkowska *et al*, 2011). Structural information has revealed that DNMT3A and DNMT3B are closely related and that both contain a PWWP domain, a PHD-like ADD domain and a catalytic domain. By contrast, DNMT3L only has an ADD domain (Fig 1A; Cheng & Blumenthal, 2010; Goll & Bestor, 2005).

From *in vitro* studies, new concepts about the inherent properties of DNMTs have been postulated. The catalytic activities of DNMT3A and DNMT3B seem to be influenced by sequences next to the target CG sites, suggesting a role for sequence preference by *de novo* DNMTs in human genomic methylation patterns (Handa & Jeltsch, 2005). Interestingly, previous *in vitro* studies reported that DNMTs methylate CA dinucleotides with high activity, in addition to being most active at CG sites (Gowher & Jeltsch, 2001). These sequence preferences could shed light on non-CG methylation,

which was recently identified in embryonic stem cells, in which CA dinucleotides are the predominant form. Although DNMT3L shows no methyltransferase activity, it is indispensable for the *de novo* methylation of most imprinted loci in germ cells (Jia *et al*, 2007). DNMT3L both stabilizes the conformation of the active-site loop of DNMT3A, to enhance *de novo* methylation, and increases the binding of SAM (Jia *et al*, 2007). Furthermore, the DNMT3A–DNMT3L complex favours CG sites that are regularly distributed at distances of 8–10 base pairs, which is in line with recent genome-wide findings of methylated CG and non-CG sites at a periodicity of 8–10 base pairs (Lister *et al*, 2009).

Chromatin-mediated mechanisms

In mouse embryonic stem cells, DNMT3L interacts with cores histones. Peptide studies have revealed that the ADD domain of DNMT3L selectively binds to the unmethylated Lys4 of histone H3 (H3K4), thereby triggering *de novo* DNA methylation at nucleosomes depleted of H3 methylated at Lys4 (Fig 1B; Ooi *et al*, 2007). Other *in vitro* studies have found that DNMT3A and DNMT3B bind to unmethylated H3K4 through their ADD domain in the absence of DNMT3L (Otani *et al*, 2009; Zhang *et al*, 2010). To understand the impact of these interactions, more studies are needed that link them to physiological contexts. Evidence is emerging about the *in vivo* regulation of DNMT3 enzyme levels and their *de novo* methylation activity. For instance, DNMT3A and DNMT3B have been mostly found to be stably anchored to nucleosomes containing higher levels of methylated DNA in somatic cells (Jeong *et al*, 2009). This nucleosome-linked DNA methylation was recently shown to be essential for regulating cellular levels of DNMT3A and DNMT3B (Sharma *et al*, 2011).

Several reviews have emphasized that DNA methylation works with local chromatin conformations to regulate specific methylation patterns that control gene transcription (Brenner & Fuks, 2007; Cedar & Bergman, 2009). Structural studies suggest that DNMTs can ‘read’ histone modifications, which leads to their recruitment to nucleosomes carrying specific marks. Genome-wide analyses have revealed that there is a strong inverse correlation between DNA methylation and histone H3K4 methylation in both embryonic stem and somatic cells (Hodges *et al*, 2009; Meissner *et al*, 2008). These findings were confirmed in postnatal neural stem cells (NSCs), for which genome-wide analysis has shown that DNMT3A is excluded from active chromatin marked by H3K4 trimethylation (H3K4me3; Wu *et al*, 2010). Interestingly, in NSCs, most DNMT3A is located within gene bodies and intergenic regions, which is consistent with the recent discovery that DNA methylation levels are high in the gene bodies of actively transcribed genes (Ball *et al*, 2009; Laurent *et al*, 2010; Weber *et al*, 2007). Other histone modifications, including H3K36me3, H3K9me3 and H3K27me3, have also been implicated in guiding DNA methylation to specific chromatin regions. Mapping studies have shown a strong correlation between DNA methylation and H3K36me3, which is primarily located in gene bodies (Hodges *et al*, 2009). Peptide studies have suggested that the DNMT3A PWWP domain specifically interacts with H3K36me3 and increases DNMT3A methylation activity on native nucleosomal DNA (Dhayan *et al*, 2010). Furthermore, differences were recently found in the levels of H3K36me3 (Kolasinska-Zwierz *et al*, 2009) and DNA methylation (Hodges *et al*, 2009; Laurent *et al*, 2010) at exon–intron boundaries, with exons being preferentially enriched for both modifications. DNA methylation has also been correlated with H3K9me3

in mammalian heterochromatin, which is typically characterized by these epigenetic modifications. Supporting data from embryonic stem cells have revealed that *de novo* DNMTs are targeted to major satellites through a DNMT3A–DNMT3B/HP1 putative complex, perhaps to reinforce the stability of the heterochromatin subdomain and genome integrity (Lehnertz *et al*, 2003).

In addition to H3K9me₃, mammalian heterochromatin is associated with the repressive mark H3K27me₃—a modification mediated by EZH2-containing polycomb repressive complexes. In some cancer cells, polycomb-mediated H3K27me₃ is associated with increased DNA methylation, and EZH2 has been proposed to mediate this crosstalk through interaction with DNMTs, leading to *de novo* methylation (Schlesinger *et al*, 2007; Vire *et al*, 2006). It will be important to elucidate the role of this repressive histone mark in targeting DNA methylation to specific genomic loci, as the EZH2–DNMT crosstalk is not observed in all cancer cells, or in normal cells or tissues.

Histone methylation patterns clearly affect *de novo* DNA methylation by guiding DNMTs to specific genomic regions. However, the key remaining questions are about the specificity of the direct interaction of DNMTs at specific loci and in response to various environmental signals. Additionally, the interplay between combinatorial histone modifications that target DNMTs across the genome awaits clarification. One possibility is that this crosstalk occurs through physical interaction between DNMTs and chromatin-modifying enzymes (Fig 1Bii; Cedar & Bergman, 2009). A recent example shows that the DNMT3A ADD domain interacts with both PRMT5 and symmetrically methylated histone H4 at Arg3 (H4R3me₂s) to silence γ -globin genes (Otani *et al*, 2009; Zhao *et al*, 2009). Although these studies improve our understanding of the way in which DNMTs are targeted to specific regions of the genome, we do not have the full picture. This suggests that as-yet-unknown factors are involved in targeting DNMTs.

RNA-based mechanisms

Plants and lower eukaryotes, such as *Schizosaccharomyces pombe* and *Caenorhabditis elegans*, have been shown to use small-interfering RNAs (siRNAs) to target silent-state epigenetic marks to specific genomic regions to mediate transcriptional gene silencing (TGS; Verdell *et al*, 2009). The DNA methylation-targeting system, termed RNA-directed DNA methylation (RdDM), involves small RNAs of 21–24 nucleotides in length, which are incorporated into an Argonaute 4 complex. The small RNAs presumably guide DRM1/2 enzymes—homologues of mammalian DNMT3A/B—to their corresponding genomic DNA (Matzke *et al*, 2009; Qi *et al*, 2006). In *Arabidopsis thaliana*, genome-wide analyses of DNA methylation at single-base resolution have shown that cellular small RNAs direct approximately 30% of cytosine methylation events (Cokus *et al*, 2008; Lister *et al*, 2008).

The recent discovery that plants and animals have highly conserved DNA methylation features (Feng *et al*, 2010) raises the question of whether RdDM is also present in mammals. Preliminary results indicate that TGS is observed when siRNAs targeted to specific gene promoters—such as ubiquitin C and human immunodeficiency virus type 1 (HIV1)—are exogenously administered to human cells (Hawkins *et al*, 2009; Turner *et al*, 2009). The mechanistic details from these studies suggest that RNA-directed TGS in human cells involves siRNAs targeted to gene promoters in an Argonaute-1-dependent manner, perhaps leading to the recruitment of DNMT3A

Fig 1 | DNMTs: structure and targeting mechanisms. (A) Schematic representation of the human DNMT1, DNMT3A, DNMT3B and DNMT3L structures with the different domains highlighted. (B) Mechanisms that target mammalian DNA methyltransferases. (i) DNMT3A and DNMT3L recognize DNA or chromatin by specific domains. The ADD domain of DNMT3A and DNMT3L was shown to interact with the unmodified Lys 4 of histone H3 (H3K4). (ii) DNMTs are recruited to DNA through protein–protein interactions with chromatin-modifying enzymes, including KMTs and HDACs. (iii) Piwi–piRNA complexes were proposed to guide DNMTs to transposon sequences. The mechanism underlying piRNA-directed DNA methylation is unknown. (iv) Interaction of pRNA with the promoter of rRNA genes might mediate the recruitment of DNMT3B and enable DNA methylation. DNMT, DNA methyltransferase; HDAC, histone deacetylase; KMT, lysine methyltransferase; piRNA, piwi-interacting RNA; pRNA, promoter-associated RNA; rRNA, ribosomal RNA.

and HDAC1-containing epigenetic silencing complexes. Attention should be given to establishing whether there are endogenous effectors of RNA-driven TGS in human cells.

A class of small ncRNAs, the Piwi-interacting RNAs (piRNAs), have shed light on the mechanism that could guide *de novo* DNA methylation to specific sequences in mammals. piRNAs are predominantly expressed in mammalian germlines and, at 26–31 nucleotides in length, they are slightly longer than siRNAs. They are characterized by their association with proteins in the Piwi subfamily of Argonaute proteins. Mouse male germ cells that are deficient in one or two of the three murine Piwi family members, *Mili* or *Miwi2*, show defective *de novo* DNA methylation of transposons. Importantly, the mutant mice show a phenotype similar to that of DNMT3L-deficient mice (Kuramochi-Miyagawa *et al*, 2008), which has led to the hypothesis that Piwi–piRNA complexes might guide DNMT3A/DNMT3B to transposon sequences, although the molecular mechanism underlying piRNA-induced DNA methylation remains unknown. An interaction between MIWI2 and DNMT3A or DNMT3B has not been detected so far, suggesting that piRNA-containing complexes do not directly bring DNMTs to their target sequences (Aravin *et al*, 2008). Instead, Piwi–piRNA complexes might first recruit chromatin-modifying enzymes to establish epigenetically stable repression marks that eventually promote *de novo* DNA methylation through the activity of the DNMT3A–DNMT3L complex (proposed model in Fig 1Biii; Aravin *et al*, 2008).

Intriguing preliminary studies *in vitro* have shown that DNMT3A and DNMT3B are able to form RNA protein complexes (RNP; Jeffery & Nakielnny, 2004). Moreover, DNMT3A has been shown to interact with small ncRNAs (Weinberg *et al*, 2006). A recent study of the role of promoter-associated RNA (pRNA)—a ncRNA that is complementary to the promoter of ribosomal RNA (rRNA) genes—reported the involvement of DNMT3B in the transcriptional silencing of rRNA genes in mouse embryonic fibroblasts. A direct interaction between pRNA and the T₀ element located in the promoter of rRNA genes was shown to form a DNA:RNA triplex *in vitro* that is specifically recognized by DNMT3B (Fig 1Biv; Schmitz *et al*, 2010). More research is required to show an interaction between DNMTs and RNA molecules *in vivo*, and to estimate the extent of pRNA-mediated targeting of DNMTs. It will be crucial to determine whether this mechanism applies to any gene that is silenced by DNA methylation. These *in vitro* studies, together with the Piwi–piRNA studies, suggest that RNA has an important role in guiding mammalian DNMTs to specific genomic loci and, consequently, in establishing specific DNA methylation patterns. However, gaps remain in our understanding of the function and biological consequences of mammalian RdDM in the context of development and disease. With the advent of new sequencing technologies, it is clear that most of the mammalian genome is transcribed, which generates a range of RNAs with no coding capacity. It is therefore possible that several other types and classes of both small and long ncRNAs that are associated with epigenetic mechanisms will be discovered in the near future. Some of these ncRNAs might be responsible for targeting the DNA methylation machinery to the recently identified extensive gene-body methylation in mammals (Sidebar Aiv).

MiRNAs: key players in regulating DNMTs?

MiRNAs are the best-known class of short ncRNAs in mammalian cells. Typically around 21 nucleotides in length, they are imperfectly aligned with the 3'UTR of target mRNAs and induce their

translational repression, deadenylation or degradation (Filipowicz *et al*, 2008). More than 1,000 miRNAs have been described in the human genome, and there is ongoing work to delineate their specific targets. Recent reports have indicated that specific miRNAs could have a role in the control of the DNA methylation machinery and, interestingly, the altered expression of some miRNAs is linked to aberrant cancer genome methylation patterns (Fig 2). Of particular interest are the miR-29 family members, including miR-29a, miR-29b and miR-29c, which have been shown to directly target DNMT3A and DNMT3B. Overexpression of the miR-29 family in lung and acute myeloid leukaemia (AML) cancer cell lines was found to downregulate both DNMTs and global DNA methylation (Fabbri *et al*, 2007; Garzon *et al*, 2009). A parallel study has further implied that miR-29b could negatively regulate DNMT3A and DNMT3B in mouse primordial germ cells and suggested an important role for these miRNAs in female gonadal development (Takada *et al*, 2009). Interestingly, overexpression of miR-29b in AML cells was shown to downregulate Sp1, a known transcription factor for DNMT1 (Garzon *et al*, 2009). This could indicate another mechanism through which miRNAs indirectly regulate DNMTs *in vivo*.

miR-148 has also been observed to target DNMT3B in HeLa cells by binding to a recognition site located in the coding region, outside the usual 3'UTR. This observation implies that miR-148 is important for regulating the abundance of the DNMT3B splice variants (Fig 2; Duursma *et al*, 2008). More recent studies have demonstrated that DNMT1 is also regulated directly by miR-148, in both human cholangiocarcinoma and systemic lupus erythematosus (SLE) cell lines (Braconi *et al*, 2010; Pan *et al*, 2010). Overexpression of miR-148 reduced DNMT1 protein levels, which in turn promoted DNA hypomethylation and increased expression of methylation-sensitive genes, such as *CD70* in CD4⁺ T cells and *Rassf1a* in cholangiocarcinoma. Surprisingly, these studies draw different conclusions regarding the way in which miR-148 targets DNMT1: the recognition region is shown to be in either the 3'UTR (Braconi *et al*, 2011) or the coding sequence (Pan *et al*, 2010). Nevertheless, the majority of miRNAs regulating DNMT1—including miR-126 and miR-152—have been shown to target the 3'UTR of DNMT1 in SLE, human cholangiocarcinoma and liver cells (Fig 2; Braconi *et al*, 2010; Huang *et al*, 2010; Pan *et al*, 2010; Zhao *et al*, 2011). An analysis of human cholangiocarcinoma xenografts with decreased miR-152 levels confirmed that miRNAs directly modulate DNMT1 *in vivo*, resulting in the silencing of crucial tumour suppressor genes (Braconi *et al*, 2010). A previously unknown mechanism through which miRNAs regulate DNMTs was revealed by experiments inhibiting Dicer, a component of the miRNA processing machinery. Dicer-deficient mouse embryonic stem cells, which contain no mature miRNAs from the miR-290 cluster, revealed downregulation of DNMT3A, DNMT3B and DNMT1—probably through the upregulation of the DNMT repressor RBL2, a target of miR-290 (Benetti *et al*, 2008; Sinkkonen *et al*, 2008). These reports emphasize the role that miRNAs might have in mediating the post-transcriptional regulation of DNMTs during disease pathogenesis and development (summarized in Fig 2). Nevertheless, further studies are essential to determine whether miRNAs are key mediators in the regulation of endogenous DNMTs, given that the miRNAs were overexpressed in almost all of the studies in which their biological impact was addressed.

In addition to miRNAs, the ubiquitously expressed HuR protein was recently shown to regulate the expression of DNMTs

Fig 2 | Post-transcriptional regulation of mammalian DNA methyltransferases. miRNAs and the HuR protein that target DNMT1 and DNMT3A/3B mRNAs are indicated. miR-148 binds to DNMT1 and DNMT3B coding regions; miR-29 family members (miR-29s) bind to the 3'UTR region of DNMT3A/3B mRNA, leading to downregulation of DNMTs. HuR protein binds to the 3'UTR region of the DNMT3B mRNA, increasing its stability. The known functional consequences of these post-transcriptional regulations are indicated. DNMT, DNA methyltransferase; HuR, Hu-antigen R; miRNA, microRNA; mRNA, messenger RNA; UTR, untranslated region.

post-transcriptionally by binding to their 3'UTR (Fig 2). HuR binds to its target mRNAs through an RNA recognition motif, and modifies their expression by altering their stability, translation or both (Kuwano *et al*, 2008). Interestingly, the binding of HuR to DNMT3B mRNA increases the expression of DNMT3B in human colorectal cancer cells by enhancing its stability, which in turn affects both global DNA methylation and DNMT3B-specific target DNA methylation levels (Fig 2; Lopez de Silanes *et al*, 2009). Thus, a scenario is emerging in which many levels of regulation are involved to ensure that DNMTs are tightly controlled during various stages of development. Importantly, post-transcriptional regulation of DNMTs has a crucial role in disease aetiology, as has been shown in various cancers in which the expression of miRNAs is altered (Fig 2).

PTMs: a dynamic interplay regulating DNMTs

Covalent post-translational modifications (PTMs) including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, ADP-ribosylation, citrullination, butyrylation, propionylation and glycosylation, provide a vast indexing potential and an expanded

function for histone and non-histone proteins. Myriad studies have shown that in chromatin, PTMs have an important function in regulating gene expression. However, the functional significance of some of these modifications remains to be determined (Gardner *et al*, 2011). Biochemical studies have shown that DNMTs are also regulated by PTMs. The majority of these studies have been limited to *in vitro* assays that have given only a preliminary understanding of the role of PTMs in DNA methylation patterns. Nevertheless, it is interesting to note that acetylation, ubiquitination, SUMOylation, methylation and phosphorylation could influence the function of DNMTs; especially their catalytic properties, stability and interaction with other proteins (summarized in Fig 3). Advances in biochemical approaches and new methods are needed for a full appreciation of the way in which PTMs could affect DNMT function *in vivo* (Sidebar Ai).

The post-translational modification of a growing number of nuclear proteins by the small ubiquitin-like modifier SUMO-1 has emerged as an important mechanism for regulating protein–protein interaction, enzyme activity, protein stability and subcellular localization (Verger *et al*, 2003). SUMOylation of both DNMT3A and

Fig 3 | Post-translational regulation of mammalian DNA methyltransferases. A summary of the covalent PTMs of DNMT1 and DNMT3A/3B proteins highlighting their biological significance in physiological contexts. These modifications include phosphorylation (P), methylation (Me), sumoylation (SUMO), acetylation (Ac) and ubiquitination (Ub). The enzymes reported to mediate these PTMs are shown. DNMT, DNA methyltransferase; HDAC, histone deacetylase; KDM, lysine demethylase; KMT, lysine methyltransferase; LSD, lysine-specific demethylase 1; PCNA, proliferating cell nuclear antigen; PTM, post-translational modification; SUMO, small ubiquitin-like modifier UHRF1, ubiquitin-like, containing PHD and RING domains 1.

DNMT3B has been known for some time, but the endogenous modification of these DNMTs and the enzyme involved remains to be discovered. Initial reports suggest that SUMOylation of DNMT3A modifies its ability to interact with other proteins such as HDACs (Fig 3, SUMO-1 is shown as an orange circle; Kang *et al*,

2001; Ling *et al*, 2004). Although endogenous human DNMT1 is SUMOylated (Lee & Muller, 2009), the way in which this stimulates the methylation activity of DNMT1 is unclear (Sidebar Ai). To appreciate the functions of SUMO in regulating DNA methylation *in vivo*, a comprehensive analysis of SUMOylated DNMTs under

various conditions is necessary. A recent report in plants described a robust method using in-depth tandem mass spectrometry analyses to identify a large collection of endogenous SUMOylated proteins. This method could be applied to mammalian cells (Miller *et al*, 2010).

Lysine methylation has also been identified as a reversible, post-translational modification of DNMTs. Wang *et al* (2009) have suggested that methylation of DNMT1 at Lys 1096 (Lys 1094 in human DNMT1) is dynamically regulated by the lysine methyltransferase Set7/9 (also known as KMT7) and the lysine-specific demethylase 1 (LSD1, also known as KDM1; Fig 3, pink circle shows lysine methylation), which in turn affects global DNA methylation levels in mouse embryonic stem cells. In a parallel publication by Estève *et al* (2009), SET7 was shown to methylate Lys 142 of human DNMT1, and knockdown of SET7 led to increased DNMT1 levels. On the basis of reports from both groups, it seems likely that lysine methylation of DNMT1 is a signal for protein degradation through the proteasome pathway. It remains to be determined whether methylation at other sites is also involved in the regulation of DNMT1 stability, and whether DNMT3A and DNMT3B are also subject to lysine methylation. Furthermore, as the increased expression of DNMT1 is associated with many types of cancer, partly due to enhanced protein stability (Agoston *et al*, 2005), further work should evaluate the role of SET7/LSD1 in this process.

Protein phosphorylation is the most-extensively studied post-translational modification and has been shown to have a role in most, if not all, cellular processes. In particular, phosphorylation can either promote or inhibit protein–protein interactions, affect DNA binding properties and alter the subcellular localization of proteins. Surprisingly, phosphorylation of the DNA methylation machinery has not been extensively studied. DNMT1 has long been known to be phosphorylated at Ser 515 *in vitro* (Glickman *et al*, 1997). One report has suggested that phosphorylation of DNMT1 at Ser 515, located in the amino-terminal replication-foci-targeting domain, is required to maintain the interaction between the N-terminal domain of DNMT1 and its catalytic carboxy-terminal domain, which is deemed necessary for DNMT1 activity (Goyal *et al*, 2007). More recently, *in vitro* studies have identified protein kinases responsible for the phosphorylation of DNMT1. CDKL5 was found to directly bind to and weakly phosphorylate DNMT1 at its N-terminal domain (Kameshita *et al*, 2008), but the physiological significance of this phosphorylation is unclear. Recombinant CK1δ was also shown to bind to the N-terminal regulatory domain of DNMT1 and to phosphorylate Ser 146 in mouse-brain extract, thereby reducing the DNA-binding activity of DNMT1 (Sugiyama *et al*, 2010). In human cells, AKT and PKC protein kinases were shown to phosphorylate recombinant DNMT1 at residues Ser 127/143 and Ser 127, respectively (Fig 3, phosphorylation is shown as a yellow circle; Hervouet *et al*, 2010). Moreover, AKT1-mediated phosphorylation of DNMT1 at Ser 143 was shown to contribute to the stability of DNMT1 in a cell-cycle-dependent manner (Estève *et al*, 2011). Phosphorylation of DNMT1 at these sites decreased the ability of the protein to interact with PCNA and UHRF1, which are involved in the maintenance of DNA methylation patterns through the recruitment of DNMT1 during mitosis. There are no reports of the phosphorylation of the DNMT3 family of proteins so far. However, there is emerging evidence to suggest that the kinase CK2 phosphorylates DNMT3A, leading to a substantial decrease in global genomic methylation levels (F. Fuks, unpublished data; Sidebar Ai). Taken together, these

Sidebar A | In need of answers

- (i) Other than SUMOylation, methylation and phosphorylation, which post-translational modifications regulate the activity of DNMTs? What are the biological consequences of the SUMOylation and phosphorylation of DNMTs? Which kinases phosphorylate the DNMT3 family?
- (ii) Methylation and phosphorylation are important for the stability and degradation of DNMT1. Is this true of all DNMTs? Which other combinations of PTMs participate in crosstalk to dynamically control DNMTs?
- (iii) How conserved is the function of RNA-mediated control of DNA methylation in plants and mammals? Does RNA-directed control of DNMTs require different factors in mammals? Is pRNA-mediated targeting of DNMTs a general mechanism *in vivo*?
- (iv) Which other ncRNAs (small RNAs and long ncRNAs) are implicated in the targeting and function of DNMTs? Do different RNA silencing pathways cooperate to regulate DNMTs?
- (v) Is there redundancy in the control of DNMT regulation and activity or are the different mechanisms operating in different cellular contexts?

data suggest that DNA methylation is also influenced by DNMT phosphorylation. However, *in vivo* results are required to substantiate their biological significance. Future research will need to address this and investigate the role of the many PTMs that modify DNMT enzymes. There is evidence of the interplay between different PTMs in the cell-cycle-dependent regulation of DNMT1 stability. This includes the ‘methylation and phosphorylation’ switch that modulates DNMT1 stability (Estève *et al*, 2011), as well as the newly defined, coordinated series of sequential PTM events involving acetylation and ubiquitination that control DNMT1 abundance (Sidebar A; Bronner, 2011; Du *et al*, 2010). Interestingly, the dynamic crosstalk between DNMT1 monomethylation of Lys 142 by SET7 and DNMT1 phosphorylation of Ser 143 by AKT kinase (Estève *et al*, 2011) emphasizes the mutually exclusive existence of these PTMs in regulating the stability and degradation of DNMT1 and thus impacting on global genome methylation. Further support for dialogue between cell-cycle-dependent post-translational events in regulating DNMT1 involves the sequential Tip60-mediated DNMT1 acetylation of Lys 173, 1113, 1115 and 1117, and DNMT1-ubiquitination by UHRF1 (Fig 3; Choudhary *et al*, 2009; Du *et al*, 2010; Kim *et al*, 2006). Tip60-mediated DNMT1 acetylation accompanied by ubiquitination leads to its degradation, which is counteracted by the presence of HDAC1 and HAUSP. In the absence of HDAC1 and HAUSP, DNA hypomethylation is observed on the imprinted H19 locus, but not across the genome or in repetitive sequences.

Given the prominent role of DNMTs during development, the cell cycle and disease stages, new methodologies will be vital to elucidate the way in which PTMs of DNMTs act together to regulate DNA methylation levels.

Outlook and perspectives

As PTMs are involved in the regulation of many proteins, we think this might be the case for DNMTs, and thereby DNA methylation. The current data suggest a role for PTMs in the regulation of DNMT levels and function, and it is crucial to determine how and which PTMs control DNMTs, and under which circumstances they do so. Thus, we urge the community to undertake the large-scale screening of PTMs in the context of the emerging methylation maps for

various genomes. Doing so will enable the creation of a comprehensive catalogue of PTMs that regulate DNMTs in various cellular contexts and phenotypes. These data will refine our understanding of the way in which such modifications influence DNA methylation in the context of regulating gene expression. Moreover, the database could be extended by adding data about the influence of PTMs on the nuclear localization of DNMTs, the interaction of DNMTs with other chromatin modifications, RNA-related processes and gene transcription. Compiling these data might allow pathways to be mapped to explain the way in which DNMTs influence DNA methylation patterns.

It is worth noting that the proteins involved in RdDM—a key mechanism for *de novo* methylation in plants—are partially homologous to mammalian DNMT3s and have conserved C-terminal catalytic domains, although the N-terminal domains are highly divergent (Sidebar Aiii). The new data described in this review concerning RNA-directed methylation in mammals suggest that DNA methylation is targeted through a base-pairing mechanism to specific regions at which sequence similarity between RNA and DNA is high. Mapping experiments are needed to analyse fully the context in which this mechanism functions during development or disease states, as well as the protein complexes involved and the chromatin modifications that participate in mediating RdDM. For instance, the mammalian genome does not seem to contain the putative SNF2 chromatin-remodelling protein DRD1 or any known homologue of RNA-dependent RNA polymerase, both of which are required for RdDM in plants. It is therefore imperative that the key mediators of this mechanism in mammals are identified, as well as the signals that trigger it under various cell conditions. Genome-wide maps of the contribution of RNA to DNA methylation patterns during development and in disease conditions would be of great interest.

The emerging picture of the recruitment of DNMTs takes into account the previously described chromatin-based mechanisms and structural properties of DNMTs, and adds the recently discovered contribution of RNAs to fine-tune the sequence preferences observed in DNA methylation patterns. Identifying the partners that function at different sequences is one of the main objectives for the future. For example, is there a coordinated crosstalk between ncRNAs and H3K36me3 in recruiting DNMTs to gene bodies? Experiments that answer these and other questions could substantiate the contribution of ncRNAs, chromatin, PTMs and the inherent properties of DNMTs in regulating DNA methylation and, consequently, gene expression (Sidebar Av). The future of the DNA methylation field lies in the comprehensive integration of studies that link genome-wide mapping of methylated cytosines with global analysis of the methylation machinery.

ACKNOWLEDGEMENTS

H.D. is supported by the Brussels Region programme 'BruBreast'. M.N.N. is supported by the European Union grant CANCELDIP FP7. F.F. is a Senior Research Associate from the Belgian Fonds de la Recherche Scientifique.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Agoston AT, Argani P, Yegnasubramanian S, De Marzo AM, Ansari-Lari MA, Hicks JL, Davidson NE, Nelson WG (2005) Increased protein stability causes DNA methyltransferase 1 dysregulation in breast cancer. *J Biol Chem* **280**: 18302–18310

Aravin AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, Toth KF, Bestor T, Hannon GJ (2008) A piRNA pathway primed by individual transposons is linked to *de novo* DNA methylation in mice. *Mol Cell* **31**: 785–799

Avvakumov GV, Walker JR, Xue S, Li Y, Duan S, Bronner C, Arrowsmith CH, Dhe-Paganon S (2008) Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. *Nature* **455**: 822–825

Ball MP, Li JB, Gao Y, Lee JH, LeProust EM, Park IH, Xie B, Daley GQ, Church GM (2009) Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat Biotechnol* **27**: 361–368

Benetti R *et al* (2008) A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. *Nat Struct Mol Biol* **15**: 998

Bestor T, Laudano A, Mattaliano R, Ingram V (1988) Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. *J Mol Biol* **203**: 971–983

Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* **16**: 6–21

Braconi C, Huang N, Patel T (2010) MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology* **51**: 881–890

Braconi C, Valeri N, Kogure T, Gasparini P, Huang N, Nuovo GJ, Terracciano L, Croce CM, Patel T (2011) Expression and functional role of a transcribed noncoding RNA with an ultraconserved element in hepatocellular carcinoma. *Proc Natl Acad Sci USA* **108**: 786–791

Brenner C, Fuks F (2007) A methylation rendezvous: reader meets writers. *Dev Cell* **12**: 843–844

Bronner C (2011) Control of DNMT1 abundance in epigenetic inheritance by acetylation, ubiquitylation, and the histone code. *Sci Signal* **4**: pe3

Cedar H, Bergman Y (2009) Linking DNA methylation and histone modification: patterns and paradigms. *Nat Rev Genet* **10**: 295–304

Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R (1998) DNA hypomethylation leads to elevated mutation rates. *Nature* **395**: 89–93

Cheng X, Blumenthal RM (2010) Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. *Biochemistry* **49**: 2999–3008

Choudhary C, Kumar C, Gnäd F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **325**: 834–840

Cokus SJ, Feng S, Zhang X, Chen Z, Merriman B, Haudenschild CD, Pradhan S, Nelson SF, Pellegrini M, Jacobsen SE (2008) Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* **452**: 215–219

Dhayalan A, Rajavelu A, Rathert P, Tamas R, Jurkowska RZ, Ragozin S, Jeltsch A (2010) The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *J Biol Chem* **285**: 26114–26120

Du Z *et al* (2010) DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. *Sci Signal* **3**: ra80

Duursma AM, Kedde M, Schrier M, le Sage C, Agami R (2008) miR-148 targets human DNMT3b protein coding region. *RNA* **14**: 872–877

Estève PO, Chin HG, Benner J, Feehery GR, Samaranyake M, Horwitz GA, Jacobsen SE, Pradhan S (2009) Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. *Proc Natl Acad Sci USA* **106**: 5076–5081

Estève PO, Chang Y, Samaranyake M, Upadhyay AK, Horton JR, Feehery GR, Cheng X, Pradhan S (2011) A methylation and phosphorylation switch between an adjacent lysine and serine determines human DNMT1 stability. *Nat Struct Mol Biol* **18**: 42–48

Fabbri M *et al* (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci USA* **104**: 15805–15810

Feng S *et al* (2010) Conservation and divergence of methylation patterning in plants and animals. *Proc Natl Acad Sci USA* **107**: 8689–8694

Filipowicz W, Bhattacharyya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* **9**: 102–114

Gardner KE, Allis CD, Strahl BD (2011) OPERating Chromatin ON, a Colorful Language where Context Matters. *J Mol Biol* **409**: 36–46

Garzon R *et al* (2009) MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. *Blood* **113**: 6411–6418

- Glickman JF, Pavlovich JG, Reich NO (1997) Peptide mapping of the murine DNA methyltransferase reveals a major phosphorylation site and the start of translation. *J Biol Chem* **272**: 17851–17857
- Goll MG, Bestor TH (2005) Eukaryotic cytosine methyltransferases. *Annu Rev Biochem* **74**: 481–514
- Gowher H, Jeltsch A (2001) Enzymatic properties of recombinant Dnm3a DNA methyltransferase from mouse: the enzyme modifies DNA in a non-processive manner and also methylates non-CpG [correction of non-CpA] sites. *J Mol Biol* **309**: 1201–1208
- Goyal R, Rathert P, Laser H, Gowher H, Jeltsch A (2007) Phosphorylation of serine-515 activates the mammalian maintenance methyltransferase Dnmt1. *Epigenetics* **2**: 155–160
- Handa V, Jeltsch A (2005) Profound flanking sequence preference of Dnmt3a and Dnmt3b mammalian DNA methyltransferases shape the human epigenome. *J Mol Biol* **348**: 1103–1112
- Hawkins PG, Santos S, Adams C, Anest V, Morris KV (2009) Promoter targeted small RNAs induce long-term transcriptional gene silencing in human cells. *Nucleic Acids Res* **37**: 2984–2995
- Hervouet E, Lallier L, Debien E, Cheray M, Geairon A, Rogniaux H, Loussouarn D, Martin SA, Vallette FM, Cartron PF (2010) Disruption of Dnmt1/PCNA/UHRF1 interactions promotes tumorigenesis from human and mice glial cells. *PLoS ONE* **5**: e11333
- Hodges E *et al* (2009) High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. *Genome Res* **19**: 1593–1605
- Howard G, Eiges R, Gaudet F, Jaenisch R, Eden A (2008) Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. *Oncogene* **27**: 404–408
- Huang J, Wang Y, Guo Y, Sun S (2010) Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology* **52**: 60–70
- Jair KW, Bachman KE, Suzuki H, Ting AH, Rhee I, Yen RW, Baylin SB, Schuebel KE (2006) *De novo* CpG island methylation in human cancer cells. *Cancer Res* **66**: 682–692
- Jeffery L, Nakielnny S (2004) Components of the DNA methylation system of chromatin control are RNA-binding proteins. *J Biol Chem* **279**: 49479–49487
- Jeong S, Liang G, Sharma S, Lin JC, Choi SH, Han H, Yoo CB, Egger G, Yang AS, Jones PA (2009) Selective anchoring of DNA methyltransferases 3A and 3B to nucleosomes containing methylated DNA. *Mol Cell Biol* **29**: 5366–5376
- Jia D, Jurkowska RZ, Zhang X, Jeltsch A, Cheng X (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for *de novo* DNA methylation. *Nature* **449**: 248–251
- Jones PA, Baylin SB (2007) The epigenomics of cancer. *Cell* **128**: 683–692
- Jurkowska RZ, Jurkowski TP, Jeltsch A (2011) Structure and function of mammalian DNA methyltransferases. *ChemBiochem* **12**: 206–222
- Kameshita I, Sekiguchi M, Hamasaki D, Sugiyama Y, Hatano N, Suetake I, Tajima S, Sueyoshi N (2008) Cyclin-dependent kinase-like 5 binds and phosphorylates DNA methyltransferase 1. *Biochem Biophys Res Commun* **377**: 1162–1167
- Kang ES, Park CW, Chung JH (2001) Dnmt3b, *de novo* DNA methyltransferase, interacts with SUMO-1 and Ubc9 through its N-terminal region and is subject to modification by SUMO-1. *Biochem Biophys Res Commun* **289**: 862–868
- Kim SC *et al* (2006) Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol Cell* **23**: 607–618
- Kolasinska-Zwiercz P, Down T, Latorre I, Liu T, Liu XS, Ahringer J (2009) Differential chromatin marking of introns and expressed exons by H3K36me3. *Nat Genet* **41**: 376–381
- Kuramochi-Miyagawa S *et al* (2008) DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes Dev* **22**: 908–917
- Kuvano Y, Kim HH, Abdelmohsen K, Pullmann R, Jr., Martindale JL, Yang X, Gorospe M (2008) MKP-1 mRNA stabilization and translational control by RNA-binding proteins HuR and NF90. *Mol Cell Biol* **28**: 4562–4575
- Laurent L *et al* (2010) Dynamic changes in the human methylome during differentiation. *Genome Res* **20**: 320–331
- Lee B, Muller MT (2009) SUMOylation enhances DNA methyltransferase 1 activity. *Biochem J* **421**: 449–461
- Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, Li E, Jenuwein T, Peters AH (2003) Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr Biol* **13**: 1192–1200
- Li E, Bestor TH, Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69**: 915–926
- Ling Y, Sankpal UT, Robertson AK, McNally JG, Karpova T, Robertson KD (2004) Modification of *de novo* DNA methyltransferase 3a (Dnm3a) by SUMO-1 modulates its interaction with histone deacetylases (HDACs) and its capacity to repress transcription. *Nucleic Acids Res* **32**: 598–610
- Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, Ecker JR (2008) Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. *Cell* **133**: 523–536
- Lister R *et al* (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462**: 315–322
- Lopez de Silanes I *et al* (2009) The RNA-binding protein HuR regulates DNA methylation through stabilization of DNMT3b mRNA. *Nucleic Acids Res* **37**: 2658–2671
- Matzke M, Kanno T, Daxinger L, Huettel B, Matzke AJ (2009) RNA-mediated chromatin-based silencing in plants. *Curr Opin Cell Biol* **21**: 367–376
- Meissner A *et al* (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* **454**: 766–770
- Miller MJ, Barrett-Wilt GA, Hua Z, Vierstra RD (2010) Proteomic analyses identify a diverse array of nuclear processes affected by small ubiquitin-like modifier conjugation in *Arabidopsis*. *Proc Natl Acad Sci USA* **107**: 16512–16517
- Miremadi A, Oestergaard MZ, Pharoah PD, Caldas C (2007) Cancer genetics of epigenetic genes. *Hum Mol Genet* **16**: R28–R49
- Ooi SK *et al* (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to *de novo* methylation of DNA. *Nature* **448**: 714–717
- Otani J, Nankumo T, Arita K, Inamoto S, Ariyoshi M, Shirakawa M (2009) Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO Rep* **10**: 1235–1241
- Pan W, Zhu S, Yuan M, Cui H, Wang L, Luo X, Li J, Zhou H, Tang Y, Shen N (2010) MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. *J Immunol* **184**: 6773–6781
- Portela A, Esteller M (2010) Epigenetic modifications and human disease. *Nat Biotechnol* **28**: 1057–1068
- Qi Y, He X, Wang XJ, Kohany O, Jurka J, Hannon GJ (2006) Distinct catalytic and non-catalytic roles of ARGONAUTE4 in RNA-directed DNA methylation. *Nature* **443**: 1008–1012
- Reik W, Dean W, Walter J (2001) Epigenetic reprogramming in mammalian development. *Science* **293**: 1089–1093
- Robertson KD (2005) DNA methylation and human disease. *Nat Rev Genet* **6**: 597–610
- Schlesinger Y *et al* (2007) Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo* methylation in cancer. *Nat Genet* **39**: 232–236
- Schmitz KM, Mayer C, Postepska A, Grumt I (2010) Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rDNA genes. *Genes Dev* **24**: 2264–2269
- Sharif J *et al* (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450**: 908–912
- Sharma S, De Carvalho DD, Jeong S, Jones PA, Liang G (2011) Nucleosomes containing methylated DNA stabilize DNA methyltransferases 3A/3B and ensure faithful epigenetic inheritance. *PLoS Genet* **7**: e1001286
- Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W (2008) MicroRNAs control *de novo* DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nat Struct Mol Biol* **15**: 259–267
- Song CX *et al* (2011) Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. *Nat Biotechnol* **29**: 68–72
- Sugiyama Y, Hatano N, Sueyoshi N, Suetake I, Tajima S, Kinoshita E, Kinoshita-Kikuta E, Koike T, Kameshita I (2010) The DNA-binding activity of mouse DNA methyltransferase 1 is regulated by phosphorylation with casein kinase 1 delta/epsilon. *Biochem J* **427**: 489–497
- Takada S, Berezikov E, Choi YL, Yamashita Y, Mano H (2009) Potential role of miR-29b in modulation of Dnmt3a and Dnmt3b expression in primordial germ cells of female mouse embryos. *RNA* **15**: 1507–1514
- Turner AM, De La Cruz J, Morris KV (2009) Mobilization-competent lentiviral vector-mediated sustained transcriptional modulation of HIV-1 expression. *Mol Ther* **17**: 360–368
- Verdel A, Vasseur A, Le Gorrec M, Touat-Todeschini L (2009) Common themes in siRNA-mediated epigenetic silencing pathways. *Int J Dev Biol* **53**: 245–257

- Verger A, Perdomo J, Crossley M (2003) Modification with SUMO: a role in transcriptional regulation. *EMBO Rep* **4**: 137–142
- Vire E *et al* (2006) The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* **439**: 871–874
- Wang J *et al* (2009) The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat Genet* **41**: 125–129
- Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schubeler D (2007) Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* **39**: 457–466
- Weinberg MS, Villeneuve LM, Ehsani A, Amarzguioui M, Aagaard L, Chen ZX, Riggs AD, Rossi JJ, Morris KV (2006) The antisense strand of small interfering RNAs directs histone methylation and transcriptional gene silencing in human cells. *RNA* **12**: 256–262
- Wu H, Coskun V, Tao J, Xie W, Ge W, Yoshikawa K, Li E, Zhang Y, Sun YE (2010) Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. *Science* **329**: 444–448
- Zhang Y *et al* (2010) Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic Acids Res* **38**: 4246–4253
- Zhao Q *et al* (2009) PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. *Nat Struct Mol Biol* **16**: 304–311

- Zhao S, Wang Y, Liang Y, Zhao M, Long H, Ding S, Yin H, Lu Q (2011) MicroRNA-126 regulates DNA methylation in CD4(+) T cells and contributes to systemic lupus erythematosus by targeting DNA methyltransferase 1. *Arthritis Rheum* **63**: 1376–1386

Matladi N. Ndlovu (left), François Fuks (middle) & Hélène Denis