CTMI (2006) 301:45–66


# DNA Methyltransferases: Facts, Clues, Mysteries

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**Abstract** DNA methylation plays a pivotal role during development in mammals and is central to transcriptional silencing. The DNA methyltransferases (DNMTs) are responsible for the generation of genomic methylation patterns leading to gene silencing, but the underlying molecular basis remains largely shrouded in mystery. Here we review our current understanding of the mechanisms by which DNMTs repress transcription and how they are targeted to preferred DNA sequences. Emerging evidence points to an essential and intricate web of interactions between DNMTs and the chromatin environment in which they function. The recent identification of novel transcription factors recruiting the DNMTs may open new avenues of research into the origin of DNA methylation patterns. Thanks to these emerging clues, researchers have begun to lift the veil on the multi-faceted DNMTs, but there remains fascinating work ahead for whoever wants to fully understand DNMTs and their role in the mammalian cell.

## 1 Introduction

DNA methylation is a major epigenetic event. It is a post-replicative, reversible, and heritable chemical modification of DNA involved in regulating a diverse range of biological processes in vertebrates, plants, and fungi. The present chapter deals mainly with DNA methylation in mammals, and particularly humans and mice.

In mammals, DNA methylation occurs predominantly at cytosine residues located within CpG dinucleotides and is associated with gene silencing. The distribution of CpG dinucleotides in the mammalian genome is uneven and non-random. Methylated DNA is most abundant in heterochromatin-containing bulk DNA such as parasitic sequences, retrotransposons, and various repeat elements. Most unmethylated CpG dinucleotides are found in "CpG islands," i.e., small stretches of CpG-rich DNA found in the 5′ regulatory regions of almost half of the genes of the genome (Bird 2002).

DNA methylation has a crucial role in normal mammalian development and plays a major role in gene expression, X-chromosome inactivation in females, and genomic imprinting. It also contributes to the stability and integrity of the genome by inactivating bulk DNA. Altered methylation patterns, with genome-wide hypomethylation and region-specific hypermethylation, are frequently found in cancers (Jones and Baylin 2002).

How does DNA methylation lead to gene silencing? How are DNA methylation patterns established and maintained? These are among the most pressing and intriguing questions in the DNA methylation field. Mechanistic insights into these questions have come from the identification and characterization of several dedicated enzymes called DNA methyltransferases (DNMTs). These key regulators of DNA methylation are the focus of this chapter, which leads the reader on a trail that starts with the structure of these proteins and progresses through the mechanisms by which they repress transcription and what we know about their targeting to preferred DNA sequences. Emphasis is laid on emerging evidence of an intimate connection between DNMTs and chromatin structure.

## 2 DNMTs: Mug Shots and Knockout

DNMTs catalyze methylation at position 5 of the cytosine ring, using *S*-adenosyl-methionine as the methyl group donor. On the basis of sequence homology, DNMTs are divided into three families: DNMT1, DNMT2, and DNMT3. This third family has three members: DNMT3A, DNMT3B, and

DNMT3L (Fig. 1). The structures and enzymatic activities of these proteins and the corresponding knockout phenotypes are reviewed in the following sections.

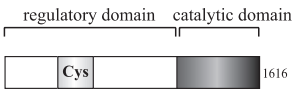
| DNMT class | Schematic structure | *Dnmt* knockout in mice | DNA methyltransferase activity |
|---|---|---|---|
| **DNMT1** |  | - Embryonic lethality (E8.5)<br>- Global hypomethylation<br>- Loss of imprinting | - ***YES***<br>- Main maintenance DNMT<br>- *de novo* activity: possible but low. |
| **DNMT2** |  | - Viable, fertile with only minor defects | - ***YES*** (low)<br>- Preference for centromeric structures |
| **DNMT3A** |  | - Postnatal lethality (4 weeks)<br>- Loss of *de novo* methylation<br>- Severe intestinal defects<br>- Impaired spermatogenesis | - ***YES***<br>- *de novo* activity; probably some maintenance activity |
| **DNMT3B** |  | - Embryonic lethality (E14.5-18.5)<br>- Loss of *de novo* methylation<br>- Mild neural tube defects<br>- Demethylation of centromeric repeat sequences | - ***YES***<br>- *de novo* activity; probably some maintenance activity<br>-Preference for minor satellite repeats |
| **DNMT3L** |  | - Viable; males are sterile (impaired spermatogenesis)<br>- Females have no viable progeniture<br>- Loss of maternal and paternal imprints in gametes | - ***NO***<br>- Cofactor of DNMT3A (enhances its *de novo* activity) |

**Fig. 1** The mammalian DNA methyltransferases (DNMTs). Three classes of DNMTs are known. Most of these proteins possess an N-terminal regulatory domain and a C-terminal catalytic domain, but DNMT2 lacks the regulatory domain and DNMT3L is catalytically inactive. Specific conserved motifs are depicted [*Cys*, cysteine-rich domain; *PHD*, plant homeodomain (ATRX-like); *PWWP*, proline- and tryptophane-rich domain]. The length of each protein is indicated in amino acids. The *third column* roughly outlines the phenotypes resulting from *Dnmt* knockout in mice. The methyltransferase activity of each DNMT (present of not; de novo and/or maintenance) is described in the *far right column*

## 2.1 DNMT Structure

A DNMT generally comprises two domains: a well-conserved catalytic domain in the carboxy-terminal part of the protein and a more variable regulatory domain in the amino-terminal region. Dnmt1 was the first enzyme to be isolated as a mammalian DNMT and the only one identified via a biochemical assay (Bestor et al. 1988; Yen et al. 1992). It has the largest amino-terminal domain of all known DNMTs. Responsible for import into the nucleus and for zinc binding, this domain also mediates protein–protein interactions.

Expression of the gene *Dnmt1* is high in proliferating cells and ubiquitous in somatic cells. During gametogenesis, expression of the gene from sex-specific promoters and 5′ exons results in sex-specific Dnmt1 isoforms whose biological functions are still quite obscure (Mertineit et al. 1998; Doherty et al. 2002). In the mouse, a Dnmt1 isoform called Dnmt1o, for "oocyte-specific," is expressed in the oocyte and pre-implantation embryo. It seems to be required only during a single S-phase in the 8-cell mouse embryo to maintain methylation patterns at imprinted loci (Howell et al. 2001).

The observation that methylation persists in mouse embryonic stem cells lacking the *Dnmt1* gene led researchers to postulate that other DNMTs must exist. Screening of expressed sequence tag (EST) databases for sequences containing motifs of the conserved catalytic domain led to the identification of three candidates: Dnmt2, Dnmt3a, and Dnmt3b (Okano et al. 1998a; Yoder and Bestor 1998).

Dnmt2 contains only the DNMT motifs; its gene is expressed, albeit to low levels, in many human and mouse tissues (Yoder and Bestor 1998). The role of this protein remains enigmatic (see Sect. 2.3).

The genes *Dnmt3a* and *Dnmt3b* show very high expression during embryogenesis and gametogenesis but much lower expression in differentiated somatic tissues. Two Dnmt3a and seven Dnmt3b isoforms have been described, featuring specific expression patterns during development and in adult tissues. Very little is known about the biological importance of individual isoforms (Okano et al. 1998a; Chen et al. 2002). To elucidate the specific function of each Dnmt3a and Dnmt3b isoform, it will be necessary to carry out genetic analyses based on isoform-specific gene disruption.

Structurally, Dnmt3a and Dnmt3b share, in addition to the catalytic site in the C-terminal region, two conserved domains in the amino-terminal region: the proline- and tryptophan-rich PWWP domain and the cysteine-rich PHD domain (for plant homeodomain).

The PWWP domain has been found in more than 60 eukaryotic proteins implicated in transcriptional regulation and chromatin organization (Stec et

al. 2000). The structure of the mouse Dnmt3b PWWP domain is known (Qiu et al. 2002). This domain probably allows targeting of Dnmt3a and Dnmt3b to pericentric heterochromatin, as it is sufficient for binding to metaphase chromosomes and promotes methylation of nucleosomal DNA (Chen et al. 2004; Ge et al. 2004). The PWWP domain of Dnmt3b binds nonspecifically to DNA (Qiu et al. 2002); that of Dnmt3a shows little DNA-binding ability (Chen et al. 2004).

The second conserved domain of the N-terminal region, the PHD domain, is conserved also in the third member of the DNMT3 family, Dnmt3L. The PHD domains of these proteins most closely resemble the imperfect PHD motif found in ATRX, a putative member of the SNF2 family of ATP-dependent chromatin remodeling proteins. A mutated ATRX gene has been found in several X-linked mental retardation disorders (Gibbons et al. 2000). The PHD domain mediates protein–protein interactions and functions as a transcriptional repressor domain (Burgers et al. 2002).

## 2.2 *Dnmt* Knockout in Mice

DNA methylation changes in a highly orchestrated way in the course of mouse development. This involves both genome-wide and gene-specific demethylation and de novo methylation (Li 2002). As mentioned above, DNA methylation is essential to mammalian development. This is vividly illustrated by targeted disruption of DNMT genes in mice, which causes embryonic (*Dnmt1* and *Dnmt3b*) or post-natal (*Dnmt3a*) mortality (Li et al. 1992; Okano et al. 1999).

*Dnmt1*$^{-/-}$ mice die around embryonic day (E)8.5, at the onset of gastrulation. Analyses of dead embryos have revealed genome-wide demethylation, biallelic expression of several (but not all) imprinted genes, and aberrant expression of Xist, a long, non-coding RNA involved in X-chromosome inactivation in females (Li et al. 1992).

*Dnmt3a*$^{-/-}$ mice die 4 weeks after birth; they display severe intestinal defects and impaired spermatogenesis. As for *Dnmt3b*$^{-/-}$ mice, they show demethylation of minor satellite DNA, mild neural tube defects, and embryo mortality at E14.5–E18.5 (Okano et al. 1999). When both *Dnmt3a* and *Dnmt3b* are disrupted in mice, doubly homozygous [*Dnmt3a*$^{-/-}$, *Dnmt3*b$^{-/-}$] embryos have a phenotype similar to that of *Dnmt1*$^{-/-}$ embryos, showing developmental arrest at the presomite stage and a distorted neural tube around E8.5 (Okano et al. 1999).

Mice with a disrupted *Dnmt2* gene are viable and fertile, with minor defects (Okano et al. 1998b). This is in agreement with results obtained on *Dnmt2*$^{-/-}$ embryonic stem (ES) cells. These cells are viable and show no obvious alter-

ation of their DNA methylation pattern (Okano et al. 1998b). As mentioned in the next section, this mild phenotype of *Dnmt2*$^{-/-}$ is probably linked to the very low enzymatic activity of the DNMT2 protein (Hermann et al. 2003).

*Dnmt3L* $^{-/-}$ mice are viable, but males are sterile and the heterozygous progeny of homozygous females die in utero and show complete loss of maternal genomic imprinting (Hata et al. 2002). This phenotype is indistinguishable from that of conditional knockout mice having a disrupted *Dnmt3a* gene in germ cells only. This highlights the crucial role of Dnmt3L and Dnmt3a in maternal imprinting (Kaneda et al. 2004). A study also suggests that Dnmt3L is an important cofactor for Dnmt3a (Chedin et al. 2002). Dnmt3L may additionally be involved in retrotransposon silencing during premeiotic genome scanning in male germ cells (Bourc'his and Bestor 2004), since deletion of *Dnmt3L* in early male germ cells prevents de novo methylation of dispersed retrotransposons and causes meiotic failure in spermatocytes.

## 2.3 DNMT Methyltransferase Activity: A Complex Issue

DNMTs have commonly been classified as either "maintenance" (DNMT1) or "de novo" (DNMT3) methyltransferases. This classification is based on the observation that Dnmt1 interacts with proliferating-cell nuclear antigen (PCNA) (Chuang et al. 1997), an auxiliary component of the DNA replication complex, and localizes to replication foci (Leonhardt et al. 1992). Yet it is emerging with increasing clarity that this classification is far too simplistic.

In human colorectal cancer cells, for example, there is evidence that DNA methylation patterns are maintained not by DNMT1 alone but by cooperation between DNMT1 and DNMT3B (Rhee et al. 2000, 2002; Ting et al. 2004). The effects of *Dnmt3a* and *Dnmt3b* disruption in ES cells likewise indicate that both Dnmt3a and Dnmt3b are involved in maintaining DNA methylation patterns (Chen et al. 2003). Dnmt1, on the other hand, shows little or no de novo methylation activity in vivo. Li and coworkers have recently proposed a model for the action of these three DNMTs (Chen et al. 2003): DNMT1 would be the main maintenance enzyme, acting with high efficiency but not full accuracy. DNMT3A and DNMT3B, via their de novo activity, would act as "proofreaders," restoring CpG methylation at sites left untouched by DNMT1.

DNMT3L shows no methyltransferase activity, but it is nevertheless involved in the regulation of DNA methylation. As mentioned above, it contributes particularly to establishing genomic imprinting during gametogenesis. It would appear to act as a cofactor for Dnmt3a, enhancing the latter's de novo activity (Bourc'his et al. 2001; Bourc'his and Bestor 2004; Kaneda et al. 2004).

Although DNMT2, as mentioned above, has retained only one of the domains characteristic of DNMTs, the methyltransferase domain, it was not shown until recently to be catalytically active (Hermann et al. 2003). It was also shown to display a certain sequence specificity for centromeric structures. This recent observation will likely revive interest in this still-mysterious member of the DNMT family.

## 3
## How Do DNMTs Interfere with Transcription?

DNMTs participate in gene silencing, but how? It has been known for many years that DNA methylation and chromatin structure are connected. In mammalian genomes, for example, high levels of DNA methylation coincide with heterochromatic regions (Razin and Cedar 1977). Also, methylated CpG islands (such as those of the female-inactivated X chromosome) appear in closed, transcriptionally silent chromatin with deacetylated histones, whereas unmethylated islands in gene promoters are transcriptionally favorable and have an open chromatin structure with highly acetylated histones (Bird and Wolffe 1999).

The mechanistic basis of the link between DNA methylation and chromatin structure has long remained obscure, but the recent explosion in knowledge on how chromatin organization modulates gene transcription has paved the way towards elucidating this link. As described below and Sect. 3.2 with special emphasis on DNMTs, it is now increasingly clear that DNA methylation and chromatin organization work hand in hand to repress gene expression.

### 3.1
### Cross-talk and Transcriptional Silencing

Initial papers from the laboratories of A. Bird and A. Wolffe were the first to unveil a mechanistic connection between DNA methylation and histone modification. They showed that methyl-CpG binding domain (MBD) proteins, which selectively recognize methylated CpG dinucleotides, are components of—or establish contacts with—histone deacetylase (HDAC) complexes (Jones et al. 1998; Nan et al. 1998). HDACs remove acetyl groups from histone tails and help to maintain nucleosomes in a compact, transcriptionally silent state.

Next, a much more direct connection between CpG methylation and deacetylation was identified: DNMTs appear to repress transcription through recruitment of histone deacetylases (Burgers et al. 2002). The fact that each

DNMT associates with HDAC prompts the question: Why is this contact necessary? One clue might lie in the ability of DNMTs to act as maintenance and/or de novo methyltransferases. A challenge for the cell is to restore in newly replicated DNA the chromatin structure needed to maintain the transcriptional activity states dictated by chromatin modifications. In the case of at least one maintenance enzyme, DNMT1, its association with HDAC is particularly attractive: It occurs predominantly at replication foci during the late S-phase, when most of the heterochromatin is duplicated (Rountree et al. 2000). DNMT1 may thus be necessary to ensure that the histones forming the nucleosomes assembled at newly replicated sites are deacetylated.

An unexpected finding has emerged from the study of the DNMT–HDAC interaction, mediated by the non-catalytic N-terminal portion of the DNMT. Intriguingly, transcriptional silencing does not require preservation of DNMT enzymatic activity. In addition, Dnmt3L can still recruit the HDAC repressive machinery despite its lack of DNMT activity (Deplus et al. 2002).

It thus seems that DNMTs can carry out some HDAC-associated functions independently of their ability to methylate CpG sites, at least in certain circumstances. Although these observations remain to be confirmed in vivo, it is tempting to speculate that DNMTs are more versatile than initially anticipated. In other words, they may be multifaceted proteins performing other functions in addition to methylation of CpG dinucleotides.

More recently, DNMTs have been implicated in another chromatin-related transcriptional repression process, involving methylation of histone H3 at lysine 9. This connection was first evidenced in the ascomycete fungus *Neurospora crassa* and in the plant *Arabidopsis thaliana* by E. Selker's and S. Jacobsen's groups, respectively (Jackson et al. 2002; Selker et al. 2003). It was shown that mutations in the genes *dim-5* of Neurospora and *kryptonite* of Arabidopsis result in loss of DNA methylation in these organisms. Excitement arose from the finding that these genes encode H3-K9 histone methyltransferases.

The mechanisms linking DNA methylation to histone methylation remain unclear and there are likely several ways that connect these two epigenetic events. In Arabidopsis, the adaptor protein LHP1 (the homolog of the mammalian heterochromatin protein 1, HP1) is not needed to maintain DNA methylation, and at least deacetylase HDA6 is instead required (Bender 2004). In Neurospora, however, the HP1 protein could be a possible link between DNA and histone methylation, since it has been shown that HP1 is required for DNA methylation (Selker et al. 2003). According to the current working model in Neurospora, methylation at H3-K9 by DIM5 would create a binding platform for HP1. This adaptor protein would then recruit the DIM2 DNMT. In this way, histone methylation would influence DNA methylation.

Might such a model also apply to mammals? Is there cross-talk in mammals between histones and DNA and are DNMTs involved? Recent studies indicate that this may well be the case. DNMTs appear to associate with histone methyltransferase activities that modify lysine 9 of H3. Interaction with the Suv39h histone methyltransferase may be involved (Fuks et al. 2003a; Lehnertz et al. 2003). Contact between DNMTs and proteins HP1α and HP1β has also been demonstrated (Fuks et al. 2003a; Lehnertz et al. 2003). Lastly, results obtained with Suv39h-double-null mouse embryonic stem cells indicate that Suv39h-mediated H3-K9 trimethylation can direct Dnmt3b to major satellite repeats present in pericentric heterochromatin (Lehnertz et al. 2003). As in the proposed Neurospora model, mammalian DNMTs might thus interact with the adaptor molecule HP1 and be present in the vicinity of chromatin-containing methylated histones, this leading to CpG methylation and gene silencing.

Because mammals possess several H3-K9 methyltransferases, the methylation "conversation" is likely to be more complex in mammals than in Neurospora. This view is supported by work on Suv39h-double-null cells, based on the use of highly specific antibodies that discriminate between H3-K9 di- and trimethylation. In this study, pericentric major satellites displayed Suv39h-dependent H3-K9 trimethylation, while centromeric minor satellites showed a "preference" for Suv39h-independent H3-K9 dimethylation (Lehnertz et al. 2003). As Dnmt3b-dependent DNA methylation at minor satellites was unimpaired in Suv39h-double-null cells, it could be that H3-K9 dimethylation catalyzed by some other enzyme, the identity of which is still unknown, is responsible for the observed targeting of Dnmt3b. Other H3-K9 methyltransferases, such as G9a (Xin et al. 2003) or SETDB1 (Ayyanathan et al. 2003) are reported to regulate DNA methylation-mediated gene silencing, but whether these enzymes associate directly with DNMTs to affect CpG methylation remains to be seen.

### 3.2
### Histone and DNA Methylation: Mutual Boosting and Feedback Loops

The observations just described suggest a straight line from H3-K9 methylation to DNA methylation. This is consistent with evidence showing that only genes silenced by other mechanisms are subject to CpG methylation, which would thus be a secondary event in gene silencing (Bird 2002). Recent data indicate that DNA methylation might in turn exert a feedback effect on lysine methylation, this leading to mutual reinforcement of these two distinct methylation layers.

Work on MBD proteins supports this view. At the site of a methylated gene that it regulates, MeCP2 was found to facilitate methylation of histone

H3 at lysine 9, likely catalyzed by the Suv39h enzyme (Fuks et al. 2003b; our unpublished data). In addition, methylation by the H3-K9 enzyme SETDB1 was shown to depend on MBD1 and on DNA methylation at specific loci (Sarraf and Stancheva 2004).

Whether DNMTs dictate histone methylation directly has not been reported to date, but work on Arabidopsis may again lead the way. Mutational analyses indicate that MET1, the plant homolog of mammalian DNMT1, influences H3-K9 methylation (Soppe et al. 2002). Thus, while much more work is needed to extend these studies to other settings, it would seem that epigenetic information, embodied in residue methylation states, can flow from histones to DNA and back. This would be similar to the established flow of information between deacetylated histones and methylated DNA, involving physical association of MBDs and DNMTs with HDACs and resulting in feedback loops (Jaenisch and Bird 2003).

All this suggests that DNA methylation may lead to gene silencing as part of an epigenetic program carried out through the interactions illustrated in Fig. 2. In the initial phase, DNMTs bound to an adaptor molecule such as HP1 would add methyl groups to DNA only on chromatin that is methylated at lysine 9 of histone H3. Association of the DNMTs with an H3-H9 methyltransferase (e.g., Suv39h) would ensure a direct impact of H3-K9 methylation states on the DNMTs. These would also make contacts with HDACs. This would lead to partial gene silencing. In a second step, the generation of methylated DNA by the DNMTs would permit binding of MBDs to DNA. The bound MBDs would in turn interact with H3-K9 methyltransferase and facilitate lysine methylation. As deacetylation of histone H3 at lysine 9 is necessary for methylation to take place on this residue (Rea et al. 2000), deacetylation of histone H3 at lysine 9 would be followed by histone methylation, which in turn might result in the recruitment of proteins such as HP1.

It will be essential in the future to unravel in more detail the precise sequence of events. Multiple mechanisms are likely to contribute to the establishment and maintenance of silenced epigenetic states. Nevertheless, the above model is attractive because it suggests that DNA methylation might act together with histone deacetylation and H3-K9 methylation to generate a self-reinforcing cycle and thereby perpetuate and maintain a repressed chromatin state.

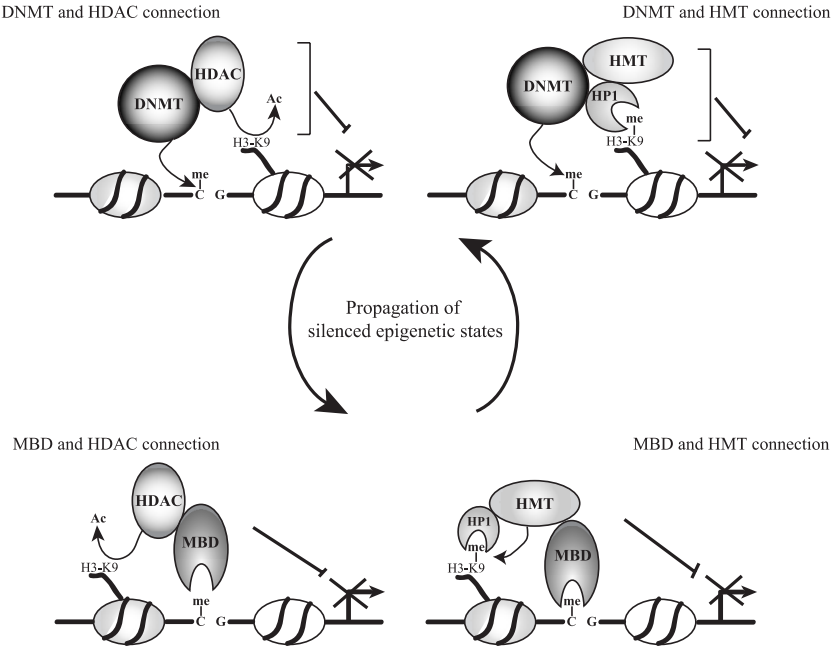



**Fig. 2** DNA methylation and chromatin modifications interact intimately to bring about transcriptional silencing. In a first phase, the association of DNMTs with HDACs leads to histone deacetylation and, in some instances at least, to CpG methylation. This would lead to chromatin compaction and transcriptional silencing. Association of DNMTs with H3-K9 histone methyltransferase (HMT) and the HP1 adaptor protein would lead to a direct impact of the H3-K9 methylation state on the DNMTs. In a second phase, methylation of CpGs by DNMTs would allow binding of methyl-CpG binding domain proteins (MBD) to the DNA. MBD would in turn associate with HDAC and the H3-K9/HP1 system and favor histone deacetylation and H3-K9 methylation, respectively. This sequential process coupling DNA methylation with histone deacetylation and H3-K9 methylation may create a self-perpetuating epigenetic cycle for the maintenance of transcriptional repression. *Ac*, acetyl group; *me*, methylated group; *H3-K9*, Lys 9 of histone H3

## 4
## How Are DNMTs Targeted to Precise DNA Sequences?

Methylated cytosines are not randomly distributed in the mammalian genome. The mechanisms underlying the establishment of DNA methylation patterns remain largely a mystery. Methylation patterns are generated by the DNMTs, and evidence is accruing that DNMTs have preferred sites of action. Targeted disruption of Dnmt3a and Dnmt3b in mouse embryonic

stem cells has demonstrated that they have some overlapping sites, while each also has its specific targets. For example Dnmt3b, but not Dnmt3a, participates in the methylation of centromeric minor satellite repeats (Okano et al. 1999). Likewise, studies on DNMT3B mutations causing a rare human condition called ICF (for immunodeficiency, centromere instability, and facial anomalies) suggest that DNMT3B methylates specific centromeric repeats (Xu et al. 1999). Experiments using a stable episomal system also show that Dnmt3a and Dnmt3b may have some distinct preferred target sites (Hsieh 1999).

How DNMT activity is preferentially targeted to specific regions of the genome is still poorly understood. DNMTs do not appear to have an intrinsic capacity to discriminate among primary nucleotide sequences. Several mechanisms, some of which are described below and Sects. 4.2 and 4.3, might explain the regional specificity that DNMTs display.

### 4.1
### Chromatin-Based Targeting

One possibility is that chromatin-modifying or -remodeling proteins might be required to attract DNMTs to DNA to be methylated. As illustrated in the previous section, emerging clues suggest that de novo DNMTs take cues from histone modifications. On the one hand, methylation at lysine 9 of H3 can facilitate CpG methylation, and DNMTs associate with H3-K9 enzymatic activity. On the other hand, DNMTs interact directly with histone deacetylases. In Neurospora, HDAC inhibition by trichostatin A (TSA) causes specific cytosine hypomethylation (Selker 1998). Moreover, transient transfection studies suggest that histone acetylation may dictate, in some instances, DNA methylation (Cervoni and Szyf 2001). The current model proposes that DNMTs might be targeted to a genomic sequence by nucleosomes featuring histone hypoacetylation or H3-K9 methylation. Thus, histone modifications would provide a basis for the generation of CpG methylation patterns by DNMTs (Fig. 3a).

In addition to histone modification, chromatin remodeling might be required for DNMT-catalyzed methylation. Emerging clues point to the possibility that chromatin remodeling might be needed to give DNMTs access to chromatin templates that would otherwise remain inaccessible. Studies on Arabidopsis, mice, and humans indicate that loss or alteration of DNA methylation may result from mutations in SNF2-like ATPases or from disruption of the corresponding genes (Meehan and Stancheva 2001), i.e., chromatin-remodeling proteins requiring ATP in order to disrupt histone–DNA interactions and to enable nucleosomes to slide along the DNA.

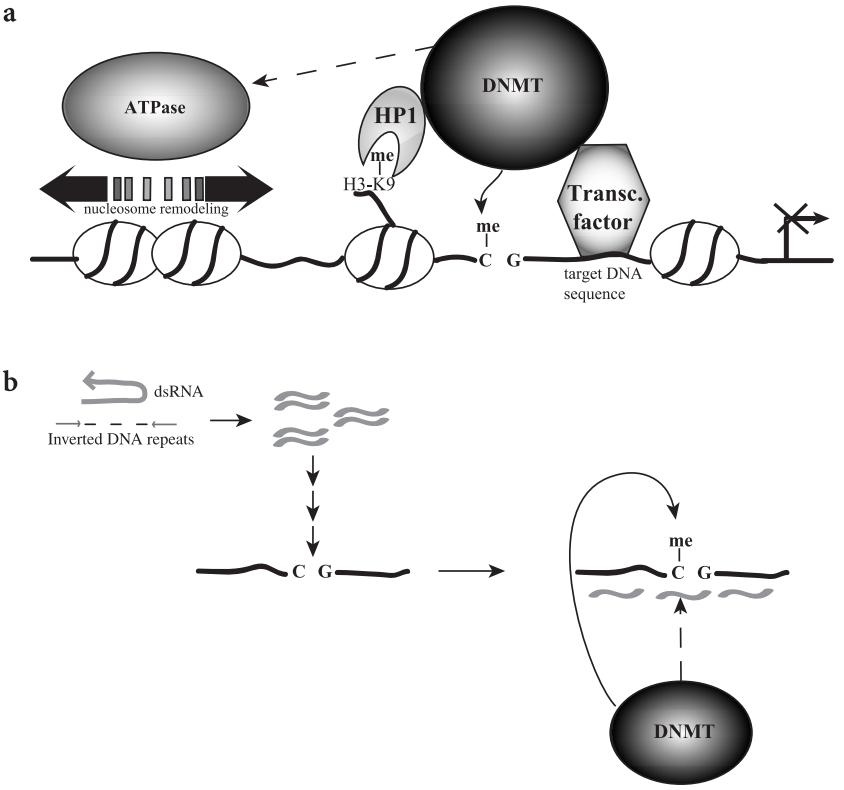



**Fig. 3a, b** Possible mechanisms for the targeting of DNMTs to specific DNA sequences. **a** Chromatin- and transcription factor-based targeting. Histone methylation at Lys 9 of H3 influences DNA methylation, possibly through recruitment of DNMTs by the adaptor HP1. HDACs associate with DNMTs (not shown) and may also provide a basis for the generation of CpG methylation patterns by DNMTs. ATP-dependent chromatin-remodeling proteins such as Lsh or ATRX could recruit DNMTs and, although this has yet to be demonstrated, might directly assist methylation of CpGs by DNMTs (*broken arrow*). Targeting of DNMTs may also be achieved through their association with specific transcription factors such as PML/RAR or Myc, with subsequent CpG methylation in the targeted promoter. **b** Do DNMTs "listen" to RNA? It seems that RNA-mediated DNA methylation (RdDM) can occur in mammals (Morris et al. 2004; Kawasaki and Taira 2004). Double-stranded (ds)RNA is processed by the Dicer enzyme into small interfering (si)RNAs. By analogy to what happens in plants, chromatin-modifying and/or -remodeling enzymes might be required for RdDM in mammals (not shown). Although this is highly speculative, RNA molecules might serve as cofactors for DNMTs. In other words, DNA methyltransferases might be recruited directly by an RNA component (*broken arrow*) to generate specific DNA methylation patterns

In mammals, the SNF2 family of ATP-dependent chromatin-remodeling proteins comprises three subfamilies: the SNF2-like, ISWI, and CHD proteins (Becker and Horz 2002). Two mammalian SNF2-family members, ATRX and Lsh, have been shown to modulate DNA methylation levels. Structurally, ATRX is most closely related to the CHD subfamily. Patients with ATRX syndrome have subtle defects in CpG methylation, including both hypo- and hypermethylation in restricted genomic regions such as ribosomal (r)DNA arrays (Meehan and Stancheva 2001). Lsh is most closely related to the ISWI subfamily of chromatin remodeling ATPases. Its targeted deletion in mice results in substantial loss of CpG methylation throughout the genome, without any observed increase in methylation (Dennis et al. 2001).

Studies on ATRX and Lsh have led to the hypothesis that genome shaping by these chromatin-remodeling proteins might be required for proper targeting of DNMTs. Improper functioning of the remodeling enzymes may lead to either hypo- or hypermethylation (as observed in ATRX patients). The latter effect may be due to aberrant targeting of DNMTs to regions that would not normally be methylated.

What could be the mechanisms by which the SNF2 ATPases alter methylation patterns? Are remodeling proteins directly contacting DNMTs to regulate their chromatin accessibility? Recent biochemical studies on DNMT3B may point to a direct connection between DNMTs and remodeling enzymes (Fig. 3a). Endogenous DNMT3B was found to associate with ATPase activity and to interact in vivo with the ATP-dependent chromatin-remodeling enzyme hSNF2H (Geiman et al. 2004). It will be crucial in the future to evaluate whether hSNF2H modulates CpG methylation patterns as observed for ATRX and Lsh.

When envisaging a potential direct link between DNMTs and SNF2 ATPases, it is necessary to consider a number of additional issues. One question is whether remodeling enzymes influence de novo DNMT activity, maintenance DNMT activity, or both. For example, work on Lsh indicates that synthesis of this protein correlates with the S-phase of the cell cycle. It has been postulated that Lsh might facilitate access of DNMTs to hemimethylated sites after replication occurs and thus contribute to maintaining methylation patterns (Dennis et al. 2001).

Another question that researchers are eager to answer is whether ATP-dependent nucleosome-remodeling enzymes can directly assist methylation of CpG residues by DNMTs. A fruitful approach might be to develop in vitro assays employing recombinant SNF2 ATPases and DNMTs with reconstituted chromatin substrates.

### 4.2
### Targeting of DNMTS by DNA-Bound Transcription Factors

Another possible mechanism for the recruitment of DNMTs to specific genome sequences might involve their association with specific transcription factors. Early work did point in this direction: It was found that DNA-binding transcriptional repressors such as E2F or RP58 can recruit DNMTs to their target promoters and thereby cause transcriptional repression (Burgers et al. 2002). Disappointingly, however, this repression was found not to depend on the methyltransferase activity of the DNMTs.

A breakthrough came from studies focusing on another transcriptional regulator, PML-RAR. This oncogenic protein, generated by a translocation, appears in acute promyelocytic leukemia. It was found that PML-RAR can recruit DNMT1 and DNMT3A to the retinoid acid receptor (RAR)β promoter, this leading to hypermethylation of the promoter and to gene silencing (Di Croce et al. 2002). This was the first demonstration that DNMTs can be recruited by a DNA-bound transcriptional repressor, with subsequent CpG methylation of the targeted-promoter.

It is tempting to draw a parallel between the targeting of DNMTs to promoters by specific DNA-binding proteins and the mechanisms by which chromatin-modifying enzymes regulate gene expression by establishing local changes in chromatin structure. For instance, histone-modifying enzymes such as acetylases and deacetylases are targeted to promoters via their association with DNA-bound activators or repressors, and this appears as a general strategy for delivering the corresponding enzymatic activities to specific promoters (Kurdistani and Grunstein 2003). By analogy, cells might use a similar general strategy to target DNMTs to precise loci.

How general a mechanism is DNMT targeting by transcription factors? Recent work in our laboratory shows that the Myc transcription factor associates in vivo with Dnmt3a and targets its enzymatic activity—through the DNA-binding protein Miz-1—to the p21Cip1 promoter. In this system, DNA methylation is required for Myc-mediated repression of p21Cip1 (Brenner et al. 2005). What's more, yeast-two hybrid screens using DNMTs as baits have led to the identification of known transcription factors that could potentially target their activity to specific promoters (our unpublished data). Thus, it is reasonable to hypothesize that DNMT-catalyzed CpG methylation steered by sequence-specific binding proteins may be a general mechanism for the establishment of DNA methylation patterns (Fig. 3a).

## 4.3 The RNA Trigger

Another potential mechanism for the establishment of DNA methylation patterns in mammals could involve RNA. This exciting possibility is attracting more and more attention. It is known that in plants, post-transcriptional gene silencing—which resembles RNA interference (RNAi)—triggers DNA methylation. RNAi is activated by the expression of dsRNA, which provides a trigger for the degradation of transcripts with which it shares sequence identity. siRNAs 21–26 nucleotides in length are key actors in RNAi, deriving from dsRNA through the action of the RNase III Dicer enzyme (Matzke and Birchler 2005).

Promoter sequence-containing dsRNA can cause gene silencing by DNA methylation of the homologous promoter regions. This RNA-directed DNA methylation (RdDM) is highly sequence-specific and largely confined to regions of RNA–DNA sequence homology (Matzke and Birchler 2005). With the help of molecular genetics, investigators are beginning to unravel the mechanisms underlying RdDM in plants. These studies reveal that RdDM requires various proteins: RNAi-pathway proteins, a novel remodeling enzyme, and also histone-modifying enzymes and DNMTs. Although RdDM seems to be a common and general mechanism for silencing gene transcription in plants, this is likely not the case in Neurospora. Notably, DNA methylation occurs normally in the latter organism in the absence of key elements of the RNA-silencing machinery (Freitag et al. 2004).

Clearly, RdDM is not a general DNA methylation-targeting mechanism. This prompts several questions: Does RNA-directed DNA methylation mechanism exist in mammals? If so, does it involve DNMTs and what is their role? It has long been known that in mammals, non-coding RNAs are involved in processes such as allelic imprinting and X inactivation. For instance, studies on mice have shown that expression of *Xist*, a non-coding RNA involved in X inactivation, is regulated by expression of its antisense RNA *Tsix*, driven by a promoter downstream from the *Xist* gene (Lee and Lu 1999). Also, an RNA component is required to maintain the structure of mouse pericentromeric heterochromatin (Maison et al. 2002). Furthermore, studies focusing on rearrangement of the α-globin gene in a patient with α-thalassemia showed that the α-globin gene on the rearranged chromosome was intact but silenced epigenetically through convergent transcripts correlating with DNA methylation (Tufarelli et al. 2003). As yet, however, there is no clear evidence that these chromatin-based regulations involve RNA-directed silencing.

More recent work has yielded a confused picture regarding the involvement of RNA-mediated CpG methylation in mammals. Studies on mouse oocytes

suggest that dsRNA expression, while inducing post-transcriptional silencing by RNAi, does not induce sequence-specific methylation of the cognate DNA sequence (Svoboda et al. 2004). Limitations to this study were that the system used was confined to a specific cell type and that RdDM targeting was analyzed in a single intronless endogenous gene. Two other reports suggest, on the contrary, that RNA-mediated DNA methylation can occur in mammals. In one study on human kidney cells, siRNA targeted to a promoter by means of lentiviral transduction was found to silence the endogenous EF1A gene, silencing being associated with DNA methylation (Morris et al. 2004). In another work, synthetic siRNAs targeted to the E-cadherin gene in human breast epithelial cells caused its transcriptional repression (Kawasaki and Taira 2004). Studies in which expression of DNMT genes was suppressed by means of siRNAs targeting the corresponding messenger (m)RNAs have shown that DNMT1 and DNMT3B, but not DNMT2, are likely necessary for siRNA-mediated transcriptional silencing of expression from the E-cadherin promoter. Bisulfite sequencing revealed a correlation between E-cadherin silencing correlates and sequence-specific CpG methylation (Kawasaki and Taira 2004). Thus, RdDM appears also to occur in mammals. Yet from the few reports available to date, it would already seem that induction of DNA methylation by siRNA in mammalian cells is not a general phenomenon. If it turns out to occur in mammals in a limited range of situations, it will be important to determine which situations, and to explain why only some cells or some genes are susceptible to RdDM. It will also be essential to unravel the underlying mechanisms. Key questions will be: How are siRNAs guided to genomic DNA? How do they gain access to it? Also worthy of special attention, given the mechanism of RdDM in plants, will be the role played by chromatin-modifying and -remodeling enzymes and the sequence of events leading to siRNA-directed DNA methylation.

Regarding DNMTs, it will be important to determine how they are mechanistically connected to the RNAi machinery. While these are still early days, one might imagine, for instance, that RNA molecules serve as cofactors for DNMTs, thereby guiding CpG methylation to precise sequences (Fig. 3b). The recent observation that DNMT3A and DNMT3B can interact, at least in vitro, with RNA molecules is intriguing (Jeffery and Nakielny 2004). Hence, although highly speculative, the possibility that DNMTs might be targeted directly by an RNA component to establish specific DNA methylation patterns may deserve future study.

## 5 Conclusions

Since the isolation and characterization of the DNMTs in the 1990s, abundant evidence has established their role as key regulators of DNA methylation. What is changing is our idea of how DNMTs cause transcriptional repression and our understanding of how chromatin structure is regulated. It seems almost certain that chromatin modifications and DNMTs are tightly linked in mammals. As discussed here, clues are emerging that DNMTs may act together with histone deacetylation and H3-K9 methylation to generate a self-reinforcing cycle that perpetuates and maintains a repressed chromatin state. Despite rapid growth of knowledge on the intimate link between chromatin and DNMTs, the picture is still blurred. It will be a notable challenge to untangle the mutual reinforcements of repression and the different states of chromatin- and DNA-modifying activities required to silence different genomic regions (e.g., highly repetitive elements versus single-copy genes). What's more, the observation that DNMTs may also silence gene expression by recruiting histone deacetylase and H3-K9 methyltransferase rather than through their ability to methylate CpG sites had led to the tempting speculation that DNMTs might be multifaceted proteins with broader roles in transcriptional repression than first anticipated.

The origin of DNA methylation patterns is a longstanding mystery. Recent studies are providing clues that may help explain how DNMTs are targeted to preferred genomic loci. Like chromatin-modifying enzymes (e.g., HDAC), DNMTs are recruited to promoters by repressors of transcription, this leading to gene silencing. We anticipate a flurry of research aiming to identify transcription factors capable of targeting DNMTs to specific genes. If this mechanism of DNMT targeting turns out to be general, a key issue will be to understand precisely how specificity is achieved with respect to the DNMT-recruiting transcription factor.

Finally, exciting new evidence suggests a connection between RNAi-mediated pathways and DNA methylation in mammals. Whether DNMTs "listen" directly to RNA remains an open question. Work shedding light on this question is eagerly awaited.

**Acknowledgements** We thank Luciano Di Croce for critical comments on the manuscript. C.D. was funded by a grant from the Belgian "Télévie-F.N.R.S". F.F. is a "Chercheur Qualifié du F.N.R.S" from the Belgian Fonds National de la Recherche Scientifique.

## References

Ayyanathan K, Lechner MS, Bell P, Maul GG, Schultz DC, Yamada Y, Tanaka K, Torigoe K, Rauscher FJ 3rd (2003) Regulated recruitment of HP1 to a euchromatic gene induces mitotically heritable, epigenetic gene silencing: a mammalian cell culture model of gene variegation. Genes Dev 17:1855–1869

Becker PB, Horz W (2002) ATP-dependent nucleosome remodeling. Annu Rev Biochem 71:247–273

Bender J (2004) Chromatin-based silencing mechanisms. Curr Opin Plant Biol 7:521–526

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

Bird AP, Wolffe AP (1999) Methylation-induced repression—belts, braces, and chromatin. Cell 99:451–454

Bourc'his D, Bestor TH (2004) Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. Nature 431:96–99

Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH (2001) Dnmt3L and the establishment of maternal genomic imprints. Science 294:2536–2539

Brenner C, Deplus R, Didelot C, Loriot A, Vire E, De Smet C, Gutierrez A, Danovi D, Bernard D, Boon T, Giuseppe Pelicci P, Amati B, Kouzarides T, de Launoit Y, Di Croce L, Fuks F (2005) Myc represses transcription through recruitment of DNA methyltransferase corepressor. EMBO J 24:336–346

Burgers WA, Fuks F, Kouzarides T (2002) DNA methyltransferases get connected to chromatin. Trends Genet 18:275–277

Cervoni N, Szyf M (2001) Demethylase activity is directed by histone acetylation. J Biol Chem 276:40778–40787

Chedin F, Lieber MR, Hsieh CL (2002) The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. Proc Natl Acad Sci U S A 99:16916–16921

Chen T, Ueda Y, Xie S, Li E (2002) A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active de novo methylation. J Biol Chem 277:38746–38754

Chen T, Ueda Y, Dodge JE, Wang Z, Li E (2003) Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. Mol Cell Biol 23:5594–5605

Chen T, Tsujimoto N, Li E (2004) The PWWP domain of Dnmt3a and Dnmt3b is required for directing DNA methylation to the major satellite repeats at pericentric heterochromatin. Mol Cell Biol 24:9048–9058

Chuang LS, Ian HI, Koh TW, Ng HH, Xu G, Li BF (1997) Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. Science 277:1996–2000

Dennis K, Fan T, Geiman T, Yan Q, Muegge K (2001) Lsh, a member of the SNF2 family, is required for genome-wide methylation. Genes Dev 15:2940–2944

Deplus R, Brenner C, Burgers WA, Putmans P, Kouzarides T, de Launoit Y, Fuks F (2002) Dnmt3L is a transcriptional repressor that recruits histone deacetylase. Nucleic Acids Res 30:3831–3838

Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, Fuks F, Lo Coco F, Kouzarides T, Nervi C, Minucci S, Pelicci PG (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 295:1079–1082

Doherty AS, Bartolomei MS, Schultz RM (2002) Regulation of stage-specific nuclear translocation of Dnmt1o during preimplantation mouse development. Dev Biol 242:255–266

Freitag M, Lee DW, Kothe GO, Pratt RJ, Aramayo R, Selker EU (2004) DNA methylation is independent of RNA interference in Neurospora. Science 304:1939

Fuks F, Hurd PJ, Deplus R, Kouzarides T (2003a) The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 31:2305–2312

Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T (2003b) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J Biol Chem 278:4035–4040

Ge YZ, Pu MT, Gowher H, Wu HP, Ding JP, Jeltsch A, Xu GL (2004) Chromatin targeting of de novo DNA methyltransferases by the PWWP domain. J Biol Chem 279:25447–25454

Geiman TM, Sankpal UT, Robertson AK, Zhao Y, Robertson KD (2004) DNMT3B interacts with hSNF2H chromatin remodeling enzyme, HDACs 1 and 2, and components of the histone methylation system. Biochem Biophys Res Commun 318:544–555

Gibbons RJ, McDowell TL, Raman S, O'Rourke DM, Garrick D, Ayyub H, Higgs DR (2000) Mutations in ATRX, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. Nat Genet 24:368–371

Hata K, Okano M, Lei H, Li E (2002) Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. Development 129:1983–1993

Hermann A, Schmitt S, Jeltsch A (2003) The human Dnmt2 has residual DNA-(cytosine-C5) methyltransferase activity. J Biol Chem 278:31717–31721

Howell CY, Bestor TH, Ding F, Latham KE, Mertineit C, Trasler JM, Chaillet JR (2001) Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. Cell 104:829–838

Hsieh CL (1999) In vivo activity of murine de novo methyltransferases, Dnmt3a and Dnmt3b. Mol Cell Biol 19:8211–8218

Jackson JP, Lindroth AM, Cao X, Jacobsen SE (2002) Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature 416:556–560

Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33 Suppl:245–254

Jeffery L, Nakielny S (2004) Components of the DNA methylation system of chromatin control are RNA-binding proteins. J Biol Chem 279:49479–49487

Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genet 3:415–428

Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 19:187–191

Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H (2004) Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. Nature 429:900–903

Kawasaki H, Taira K (2004) Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. Nature 431:211–217

Kurdistani SK, Grunstein M (2003) Histone acetylation and deacetylation in yeast. Nat Rev Mol Cell Biol 4:276–284

Lee JT, Lu N (1999) Targeted mutagenesis of Tsix leads to nonrandom X inactivation. Cell 99:47–57

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

Leonhardt H, Page AW, Weier HU, Bestor TH (1992) A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. Cell 71:865–873

Li E (2002) Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 3:662–673

Li E, Bestor TH, Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 69:915–926

Maison C, Bailly D, Peters AH, Quivy JP, Roche D, Taddei A, Lachner M, Jenuwein T, Almouzni G (2002) Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. Nat Genet 30:329–334

Matzke MA, Birchler JA (2005) RNAi-mediated pathways in the nucleus. Nat Rev Genet 6:24–35

Meehan RR, Stancheva I (2001) DNA methylation and control of gene expression in vertebrate development. Essays Biochem 37:59–70

Mertineit C, Yoder JA, Taketo T, Laird DW, Trasler JM, Bestor TH (1998) Sex-specific exons control DNA methyltransferase in mammalian germ cells. Development 125:889–897

Morris KV, Chan SW, Jacobsen SE, Looney DJ (2004) Small interfering RNA-induced transcriptional gene silencing in human cells. Science 305:1289–1292

Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393:386–389

Okano M, Xie S, Li E (1998a) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet 19:219–220

Okano M, Xie S, Li E (1998b) Dnmt2 is not required for de novo and maintenance methylation of viral DNA in embryonic stem cells. Nucleic Acids Res 26:2536–2540

Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99:247–257

Qiu C, Sawada K, Zhang X, Cheng X (2002) The PWWP domain of mammalian DNA methyltransferase Dnmt3b defines a new family of DNA-binding folds. Nat Struct Biol 9:217–224

Razin A, Cedar H (1977) Distribution of 5-methylcytosine in chromatin. Proc Natl Acad Sci U S A 74:2725–2728

Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, Jenuwein T (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406:593–599

Rhee I, Jair KW, Yen RW, Lengauer C, Herman JG, Kinzler KW, Vogelstein B, Baylin SB, Schuebel KE (2000) CpG methylation is maintained in human cancer cells lacking DNMT1. Nature 404:1003–1007

Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, Baylin SB, Vogelstein B (2002) DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 416:552–556

Rountree MR, Bachman KE, Baylin SB (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. Nat Genet 25:269–277

Sarraf SA, Stancheva I (2004) Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol Cell 15:595–605

Selker EU (1998) Trichostatin A causes selective loss of DNA methylation in Neurospora. Proc Natl Acad Sci U S A 95:9430–9435

Selker EU, Tountas NA, Cross SH, Margolin BS, Murphy JG, Bird AP, Freitag M (2003) The methylated component of the Neurospora crassa genome. Nature 422:893–897

Soppe WJ, Jasencakova Z, Houben A, Kakutani T, Meister A, Huang MS, Jacobsen SE, Schubert I, Fransz PF (2002) DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in Arabidopsis. EMBO J 21:6549–6559

Stec I, Nagl SB, van Ommen GJ, den Dunnen JT (2000) The PWWP domain: a potential protein-protein interaction domain in nuclear proteins influencing differentiation? FEBS Lett 473:1–5

Svoboda P, Stein P, Filipowicz W, Schultz RM (2004) Lack of homologous sequence-specific DNA methylation in response to stable dsRNA expression in mouse oocytes. Nucleic Acids Res 32:3601–3606

Ting AH, Jair KW, Suzuki H, Yen RW, Baylin SB, Schuebel KE (2004) CpG island hypermethylation is maintained in human colorectal cancer cells after RNAi-mediated depletion of DNMT1. Nat Genet 36:582–584

Tufarelli C, Stanley JA, Garrick D, Sharpe JA, Ayyub H, Wood WG, Higgs DR (2003) Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. Nat Genet 34:157–165

Xin Z, Tachibana M, Guggiari M, Heard E, Shinkai Y, Wagstaff J (2003) Role of histone methyltransferase G9a in CpG methylation of the Prader-Willi syndrome imprinting center. J Biol Chem 278:14996–15000

Xu GL, Bestor TH, Bourc'his D, Hsieh CL, Tommerup N, Bugge M, Hulten M, Qu X, Russo JJ, Viegas-Pequignot E (1999) Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 402:187–191

Yen RW, Vertino PM, Nelkin BD, Yu JJ, el-Deiry W, Cumaraswamy A, Lennon GG, Trask BJ, Celano P, Baylin SB (1992) Isolation and characterization of the cDNA encoding human DNA methyltransferase. Nucleic Acids Res 20:2287–2291

Yoder JA, Bestor TH (1998) A candidate mammalian DNA methyltransferase related to pmt1p of fission yeast. Hum Mol Genet 7:279–284

http://www.springer.com/978-3-540-29114-5

DNA Methylation: Basic Mechanisms
Doerfler, W.; Böhm, P. (Eds.)
2006, VIII, 324 p. 24 illus., 6 illus. in color., Hardcover
ISBN: 978-3-540-29114-5