PROSPECT
Journal of Cellular Biochemistry 108:43–51 (2009)

Journal of Cellular Biochemistry

DNA Methylation-Mediated Epigenetic Control

Andrea Rottach, Heinrich Leonhardt, and Fabio Spada*

Department of Biology II and Munich Center for Integrated Protein Science CiPS^{M}, Ludwig Maximilians University Munich, 82152 Planegg-Martinsried, Germany

ABSTRACT

During differentiation and development cells undergo dramatic morphological, and functional changes without any change in the DNA sequence. The underlying changes of gene expression patterns are established and maintained by epigenetic processes. Early mechanistic insights came from the observation that gene activity and repression states correlate with the DNA methylation level of their promoter region. DNA methylation is a postreplicative modification that occurs exclusively at the C5 position of cytosine residues (5mC) and predominantly in the context of CpG dinucleotides in vertebrate cells. Here, three major DNA methyltransferases (Dnmt1, 3a, and 3b) establish specific DNA methylation patterns during differentiation and maintain them over many cell division cycles. CpG methylation is recognized by at least three protein families that in turn recruit histone modifying and chromatin remodeling enzymes and thus translate DNA methylation into repressive chromatin structures. By now a multitude of histone modifications have been linked in various ways with DNA methylation. We will discuss some of the basic connections and the emerging complexity of these regulatory networks. J. Cell. Biochem. 108: 43–51, 2009.

KEY WORDS: DNA METHYLATION; Dnmt1; Dnmt3a; Dnmt3b; METHYL-CpG BINDING PROTEIN; Uhrf1; HISTONE MODIFICATION; EPIGENETICS

During embryonic development a single cell, the zygote, gives rise to a multitude of drastically different cell types all carrying essentially the same genetic information. Biochemical processes determining transcriptionally active and silent states make it possible for the same genome to execute the many alternate expression programs that specify all the functional and structural diversity among the cell types produced during the lifespan of an organism. Canonical transcription factor networks respond to developmental signals and environmental cues and crucially contribute to initiate specific transcriptional programs. However, due to the complexity of genomic functions in eukaryotes, transcription factors are not sufficient for full establishment and long-term stability of transcriptional states. A number of additional factors and processes contribute to the setup of specific chromatin structures that in turn determine the transcriptional activity. These processes include DNA methylation, histone posttranslational modification, incorporation of specific histone variants, and chromatin remodeling. At least for DNA methylation and some histone modifications, the respective marks and associated chromatin states are inherited through successive cell generations constituting a memory system for gene expression programs. In special cases, specific epigenetic states are even inherited through the germ line from one generation of an organism to the next. As these processes affect chromatin structure leaving the underlying genomic sequence unaltered they are deemed "epigenetic" and their comprehensive makeup across the genome is generally referred to as the epigenome [Bird, 2007]. Although epigenetic marks function to stabilize transcriptional states, they and their associated chromatin states can be altered under specific conditions. Thus, epigenetic systems allow proliferating cells to preserve their identity while retaining the necessary plasticity to adapt to environmental conditions or respond to developmental signals and differentiate.

DNA methylation is the longest known and perhaps most extensively characterized epigenetic mark. We will first outline the basic features of DNA methylation and then present an overview of its intricate crosstalk with other epigenetic pathways. These complex systems show clear parallels, but also distinguishing properties in plants and animals. Here we focus on knowledge gathered from vertebrates.

THE BASICS OF DNA METHYLATION IN VERTEBRATES

DNA methylation is a postreplicative modification that occurs exclusively at the C5 position of cytosine residues (5mC) and predominantly in the context of CpG dinucleotides in vertebrates. The covalent addition of a methyl group to cytosine is catalyzed by

Grant sponsor: Deutsche Forschungsgemeinschaft (DFG); Grant numbers: SFB 684, 646, TR5.
*Correspondence to: Fabio Spada, Department of Biology II and Munich Center for Integrated Protein Science CiPSM, Ludwig Maximilians University Munich, 82152 Planegg-Martinsried, Germany. E-mail: f.spada@lmu.de
Received 25 May 2009; Accepted 27 May 2009 • DOI 10.1002/jcb.22253 •
Published online 29 June 2009 in Wiley InterScience (www.interscience.wiley.com).

DNA (cytosine-C5) methyltransferases. Vertebrate DNA methyltransferases (Dnmts; Fig. 1) contain a highly conserved catalytic domain which includes 10 sequence motives also found in prokaryotic DNA (cytosine-C5) methyltransferases [Goll and Bestor, 2005]. Therefore, it is thought that all these enzymes use the same catalytic mechanism involving substrate recognition, flipping of the target cytosine out of the DNA double helix, formation of a covalent complex with C6 position of the cytosine, transfer of the methyl group from *S*-adenosylmethionine to the activated C5 position and release of the enzyme by elimination. Apart for Dnmt2 all Dnmts comprise in addition to a C-terminal catalytic domain (CTD) also a regulatory N-terminal region (NTR) with several distinct domains.

Bioinformatic analysis suggests that Dnmt1 evolved by the fusion of at least three ancestral genes, one contributing to the CTD and two to the NTR [Margot et al., 2000]. Dnmt1 is ubiquitous and by far the most abundant Dnmt in proliferating somatic cells, interacts with the DNA replication clamp proliferating cell nuclear antigen (PCNA) throughout S phase, displays substrate preference for hemimethylated DNA and its genetic deletion results in drastic loss of DNA methylation [Leonhardt et al., 1992; Li et al., 1992; Chuang et al., 1997; Easwaran et al., 2004]. These properties constitute the basis for a major role of Dnmt1 in maintaining genomic methylation patterns through successive DNA replication cycles. The interaction of Dnmt1 with the DNA replication machinery points to a mechanism coupling replication of genetic and epigenetic information. Although this interaction likely contributes to the accurate propagation of DNA methylation patterns, it was shown to be not strictly required for maintaining global genomic methylation [Schermelleh et al., 2007; Spada et al., 2007]. Recently, the SET- and Ring-associated (SRA) domain protein Uhrf1 has emerged as an essential cofactor for the maintenance of DNA methylation. It has been shown that Uhrf1 binds hemimethylated DNA, interacts and colocalizes with Dnmt1 at replication foci and that its genetic ablation leads to remarkably similar genomic hypomethylation and developmental arrest to those observed in Dnmt1 null mice [Uemura et al., 2000; Bostick et al., 2007; Papait et al., 2007; Sharif et al., 2007]. In addition, crystallographic studies revealed that the SRA domain flips the 5mC out of the DNA double helix, a mechanism first identified with DNA methyltransferases [Arita et al., 2008; Avvakumov et al., 2008; Hashimoto et al., 2008]. Thus, it has been proposed that Uhrf1 mediates the maintenance of genomic methylation by recruiting Dnmt1 to hemimethylated CpG sites generated during DNA replication. Despite of two potential mechanisms for faithful propagation of methylation patterns (Dnmt1-PCNA and Dnmt1-Uhrf1 interactions) the overall accuracy has been estimated only around 96% (1 error for every 25 5 mCs), which is consistent with the observed maintenance of overall patterns and site by site variability even in clonal populations [Silva et al., 1993; Laird et al., 2004].

Dnmt2 comprises only a catalytic domain, shows very weak DNA methyltransferase activity and is involved in methylation of cytoplasmic $tRNA^{Asp}$ [Hermann et al., 2004; Goll et al., 2006]. However, Dnmt2 may be responsible for rare cytosine methylation at sequence contexts other than CpG [Kunert et al., 2003; Mund et al., 2004]. To date a clear phenotype after ablation or reduction of Dnmt2 levels has been shown only in zebrafish [Okano et al., 1998; Rai et al., 2007].

Dnmt3a and 3b are largely responsible for de novo establishment of genomic methylation patterns during development [Okano et al., 1999; Kaneda et al., 2004]. Dnmt3L lacks crucial catalytic motifs and is enzymatically inactive. However, Dnmt3L interacts with Dnmt3a and 3b, stimulates their catalytic activity and is essential for the establishment of maternal imprints and methylation of retrotransposable elements in the male germ line [Gowher et al., 2000;

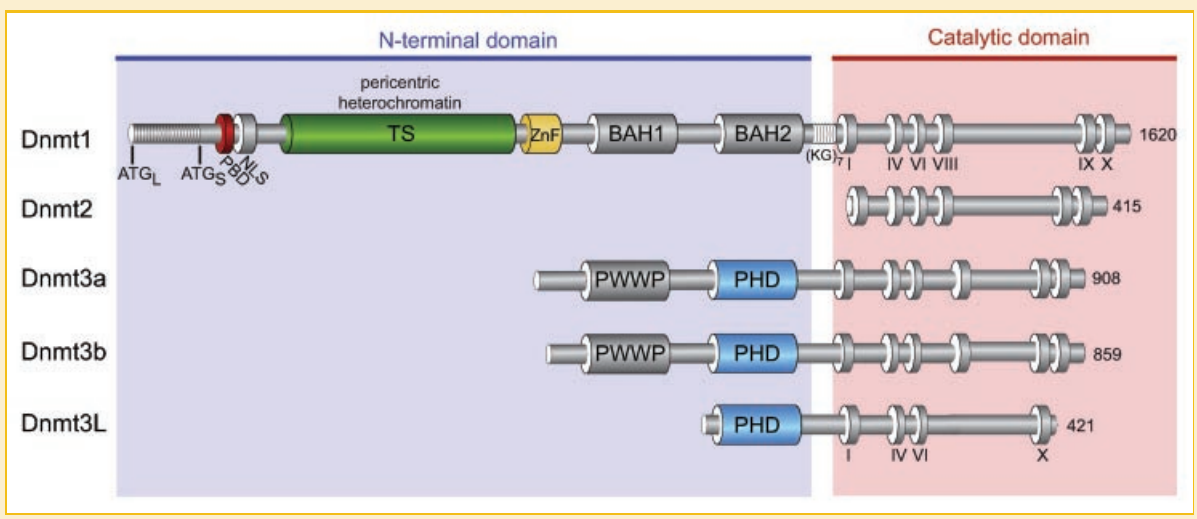

Fig. 1. Schematic representation of the mammalian DNA methyltransferase family. All Dnmts have a similar catalytic domain that features highly conserved motifs (I–X) also found in prokaryotic DNA (cytosine-5) methyltransferases. The Dnmts differ, however, in their regulatory region. Dnmt1 contains the PCNA binding domain (PBD), the pericentric heterochromatin targeting sequence (TS), a CXXC-type zinc finger motif (ZnF), and two bromo adjacent homology domains (BAH). The start codon of the long (ATG_L) and short (ATG_S) isoforms, as well as the seven lysine–glycine repeat linker (KG_7) are indicated. The regulatory domains of Dnmt3a and 3b comprise a PWWP domain named after a conserved Pro-Trp-Trp-Pro motif and a plant homeodomain (PHD).

Bourc'his et al., 2001; Hata et al., 2002; Margot et al., 2003; Bourc'his and Bestor, 2004].

A categorical distinction between maintenance Dnmt1 and de novo Dnmt3 enzymes, however, does not precisely reflect their respective functions. On one hand, Dnmt3 enzymes seem to be required for proper maintenance of DNA methylation in both somatic and embryonic stem cells (ESCs) [Liang et al., 2002; Chen et al., 2003; Dodge et al., 2005]. On the other hand, some de novo methylation was reported in ESCs lacking both Dnmt3a and 3b, although it is not clear whether this is due to the activity of Dnmt1 or Dnmt2 [Lorincz et al., 2002]. Also, direct interaction of Dnmt1 with transcription factors and its recruitment to their target sequences suggests an involvement of Dnmt1 in de novo methylation of these sequences [Robertson et al., 2000; Di Croce et al., 2002; Esteve et al., 2005]. Importantly, while the evidence for interaction and cooperation of Dnmt1 with Dnmt3 enzymes is available, the precise mechanisms, mode of targeting, and protein complex composition are unknown [Fatemi et al., 2001; Kim et al., 2002; Datta et al., 2003].

Approximately 60–70% of CpG sites are methylated in mammalian genomes. This includes all types of sequences: single copy genes and intergenic sequences as well as all kinds of repetitive elements, the latter displaying higher methylation density. Conspicuous exceptions are relatively short regions characterized by high CpG density (CpG islands) and mainly located at promoters and first exons of housekeeping genes. Nearly ubiquitous genomic methylation has been proposed as a mechanism to reduce spurious transcriptional activity (transcriptional noise) [Bird, 2002]. Promoters and enhancers with relatively low CpG density are often differentially methylated in different tissues and there is now very substantial evidence for dynamic changes of methylation patterns at these sites during cell differentiation, especially at promoters of lineage-specific and pluripotency genes [Fouse et al., 2008; Meissner et al., 2008; Mohn et al., 2008]. However, it is still debated whether the absence of DNA methylation only from selected regulatory regions is a mere consequence of transcription factor occupancy or a mechanism to favor selective binding of transcription factors to target sequences [for detailed review, see Suzuki and Bird 2008]. Nevertheless, it is generally accepted that DNA methylation marks these sequences for heritable transcriptional silencing. This forms the basis for the crucial role of DNA methylation in embryonic development, cell differentiation, neoplastic transformation, imprinting, and X chromosome inactivation [Bird, 2002]. However, as the net transcriptional state is the resultant of several interconnected epigenetic processes, cytosine methylation does not always translate in transcriptional repression [Fouse et al., 2008]. Dense methylation at repetitive elements is also thought to play a crucial role in genome stability at the level of whole organisms, as exemplified by the high tumor incidence in hypomethylated mice due to mobilization of retrotransposons and human syndromes resulting from hypomethylation of satellite repeats [Xu et al., 1999; Gaudet et al., 2003]. Surprisingly though, no major genomic alteration is apparent in cultured cells with drastically reduced or nearly no methylation [Tsumura et al., 2006; Lande-Diner et al., 2007].

Genomic methylation patterns are known to be actively erased both at specific developmental stages (e.g., demethylation of sperm chromatin upon fertilization) and during artificial reprogramming procedures such as somatic cell nuclear transfer and fusion of somatic and highly pluripotent stem cells. In vertebrates active demethylation mechanisms have long been elusive and controversial, but there is now increasing evidence for the enzymatic deamination of 5mC to thymidine followed by base or nucleotide excision repair (BER/NER) of G/T mismatches [Barreto et al., 2007; Metivier et al., 2008; Rai et al., 2008; Schmitz et al., 2009; Ma et al., 2009b]. Both Dnmt3 enzymes and cytosine deaminases of the APOBEC family have been involved in 5mC deamination, while BER is likely mediated by thymidine deglycosylases TDG and MBD4. In order to avoid deleterious accumulation of C to T transitions, these two processes seem to be tightly coupled by members of the Gadd45 protein family. Nonetheless, several important aspects remain to be defined, including whether this is the only pathway for active DNA demethylation operating in vertebrates, how many alternative and/or additional factors are involved and how the demethylation machinery is targeted to specific sequences.

MECHANISMS OF DNA METHYLATION-MEDIATED TRANSCRIPTIONAL REPRESSION AND THEIR INTERCONNECTION WITH OTHER EPIGENETIC PATHWAYS

DNA methylation-mediated transcriptional repression is thought to occur through at least two types of mechanism. The methylation mark can directly prevent the binding of transcription factors when present at their target sites, as it is the case for CTCF binding at the *H19/Igf2* imprinting control region [reviewed in Bird, 2002]. Alternatively, methylated CpG sites (mCpGs) are specifically recognized by mCpG binding proteins (MBPs) that recruit repressive chromatin modifiers and remodeling complexes. At least three types of domains and corresponding MBP families have been shown to bind mCpGs: the methyl-CpG binding domain (MBD), the UHRF, and the Kaiso protein familes (Fig. 2).

Four out of five members of the mammalian MBD family specifically bind mCpGs, the exception being MBD3 due to sequence divergence in its MBD [Hendrich and Tweedie, 2003]. Apart from the above-mentioned MBD4, all other MBDs form complexes with histone deacetylase (HDAC) and nucleosome remodeling activities (such as MeCP1 and NuRD) associated with transcriptional silencing [reviewed in Hendrich and Tweedie, 2003]. MBD1 also interacts with histone H3 lysine 9 methyltransferase (H3K9MT) SetDB1 to enforce silencing (Fig. 3A) [Sarraf and Stancheva, 2004]. Interestingly, both MBD1 and MeCP2 have been found to bind DNA and induce chromatin compaction independently of DNA methylation [Georgel et al., 2003; Jorgensen et al., 2004; Nikitina et al., 2007]. Surprisingly, a large-scale survey indicated that the majority of MeCP2 target genes in neurons are transcriptionally active [Yasui et al., 2007]. The relatively mild phenotypes of mice lacking individual MBD members have been taken to suggest a high extent of functional redundancy. However, this is in contrast with the lack of sequence and structural similarity among MBD family members

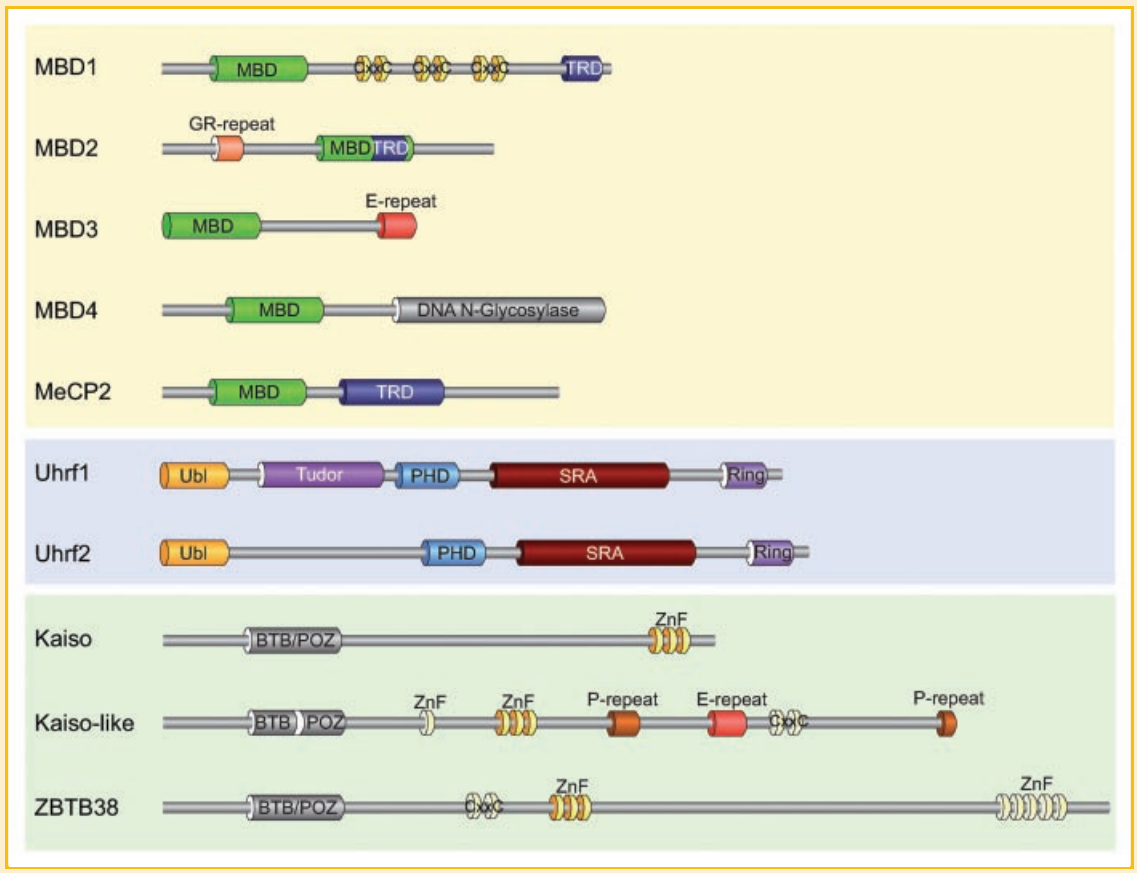

Fig. 2. The three classes of mCpG binding proteins (MBPs). The ability to recognize methylated CpG sites is mediated by different modules, the methyl-CpG binding domain (MBD), the SET- and Ring-associated (SRA) domain, or zinc finger (ZnF) motifs. MBD proteins are shaded in yellow. In addition to the MBD, MBD1, MBD2, and MeCP2 contain a trans-repressor domain (TRD). The MBD1α isoform is shown. Amino acid repeats (GR and E) are depicted in orange. Uhrf1 and the very similar Uhrf2 (shaded in blue) recognize methylated DNA via the SRA domain and contain, in addition, an Ubiquitin-like (Ubl) motif, a Tudor domain, a plant-and homeodomain (PHD), and a Ring finger. The third class of MBPs (Kaiso, Kaiso-like, and ZBTB38) is characterized by several zinc finger motifs. Binding to methylated DNA is mediated by a C2H2 zinc finger motif (yellow). The broad complex, tramtrack, and bric à brac (BTB/POZ) domain is depicted in gray.

outside the MBD. Taken together, these studies suggest that the function of MBD proteins is highly context dependent and that they are not global effectors of DNA methylation.

As mentioned above, it has been proposed that Uhrf1 contributes to the maintenance of DNA methylation patterns by recruiting Dnmt1 to asymmetrically mCpGs through its SRA domain (Fig. 3B). Uhrf1 and its homolog Uhrf2 are the only SRA domain containing proteins that have been shown to be expressed in mammalian cells. However, plants express several SRA containing proteins, including two with H3K9MT activity [Johnson et al., 2007]. Intriguingly, Uhrf1 was reported to interact with the H3K9MT G9a and HDAC1 and was involved in the silencing of tumor suppressor genes [Unoki et al., 2004; Kim et al., 2009]. Several observations suggest additional roles of Uhrf1/2 in linking CpG methylation with histone modification. Uhrf1 and 2 contain a plant homeodomain (PHD) that has been involved in binding to histone H3 and hetero-chromatin decondensation and PHD domains in other proteins can discriminate the methylation state of H3K4 [Citterio et al., 2004; Papait et al., 2008]. Available crystallographic data show a snug fit of a trimethylated H3K9 peptide in a hydrophobic cage within the tandem Tudor domain of Uhrf1 (PBD 3DB3). The Ring domain of Uhrf1 has been shown to mediate ubiquitination of histone H3 in vitro [Citterio et al., 2004]. However, the exact mechanisms and specificity of Uhrf proteins in connecting DNA methylation to repressive chromatin states are still to be resolved.

Kaiso and Kaiso-like proteins ZBTB4 and ZBTB38 share a three zinc finger motif and a broad complex, tramtrack, and bric à brac (BTB)/POZ domain at the C-terminus and are differentially expressed in mouse tissues [Yoon et al., 2003; Filion et al., 2006]. In vitro and in vivo studies showed that Kaiso binds methylated DNA through the zinc finger motif, but in contrast to the MBD and UHRF families, it requires two consecutive mCpGs for efficient binding. Biochemical analyses revealed a direct interaction of Kaiso with the repressive NCoR complex, which also contains HDAC and remodeling activities, again linking methylated DNA sequences with a deacetylated and highly structured chromatin states (Fig. 3C). In parallel with another MBD proteins, Kaiso was reported to bind a consensus sequence devoid of CpG sites, suggesting also in this case complex, context-dependent functions.

It is important to realize that in addition to DNA methylation being translated into repressive chromatin structures, DNA methylation and chromatin modification and remodeling pathways

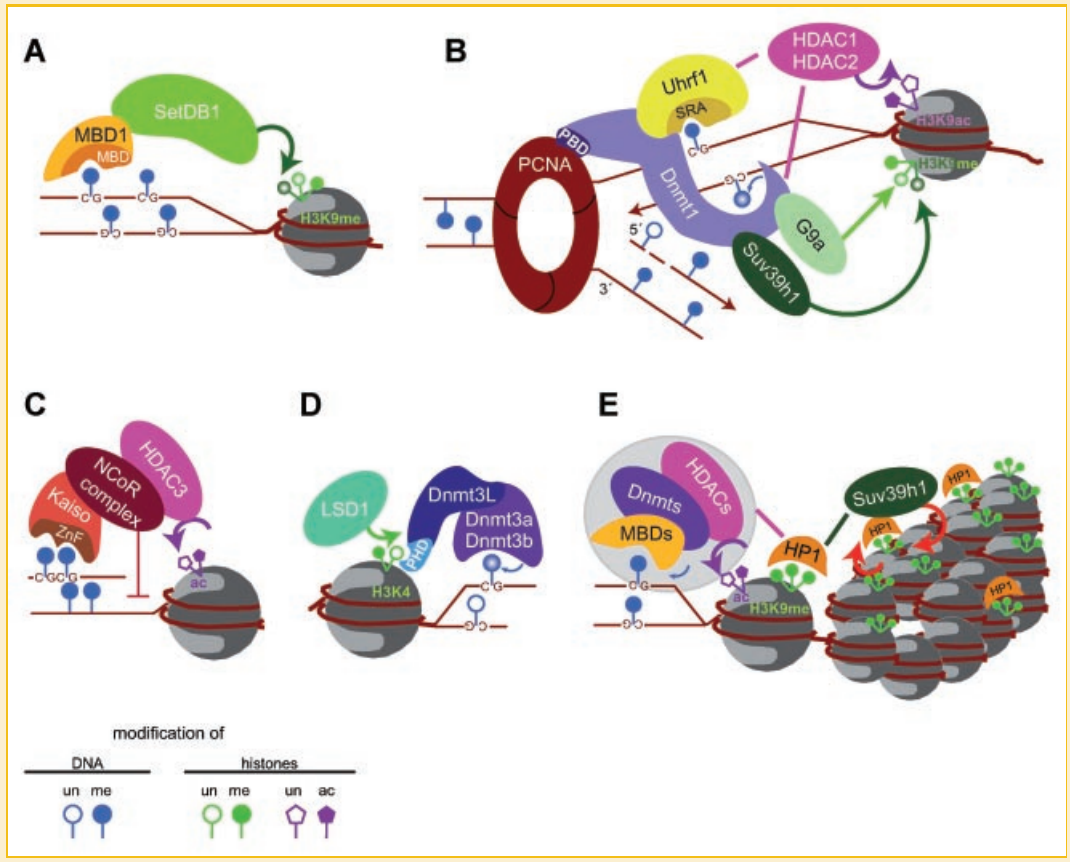

Fig. 3. Molecular links between DNA methylation, histone modification and chromatin structure. A: MBD1 binds methylated DNA via the MBD domain and recruits the lysine methyltransferase SetDB1 to enforce silencing. B: Replication-coupled maintenance of DNA methylation and histone modification. PCNA serves as a loading platform for Dnmt1 and Uhrf1. Uhrf1 recognizes hemimethylated CpG sites via the SRA domain, interacts with Dnmt1 and thus allows maintenance of genomic methylation. Interacting chromatin modifying enzymes such as HDAC1, HDAC2 (deacetylation), G9a (dimethylation of H3K9), or Suv39h1 (trimethylation of H3K9) enforce gene silencing by removing permissive acetyl-groups or introducing repressive lysine methylation on histones. C: Kaiso binds pairs of methylated CpG sites via the zinc finger motif. Interaction with the NCoR repressive complex and HDAC3 (deacetylation) promotes repression of transcription. D: De novo methylation requires the DNA methylatransferases Dnmt3a and 3b. Dnmt3L serves as a regulatory factor and via its plant homeodomain (PHD) mediates the interaction with unmethylated histone H3 lysine 4 (H3K4) generated by LSD1. E: Binding of HP1 mediates long-term silencing of chromatin regions. A positive feedback loop is created by HP1 recruiting Suv39H1 that trimethylates H3K9 generating additional binding sites for HP1.

reciprocally affect each other in multiple ways. An example is the demethylation of H3K4 by LSD1. This creates a binding site for the PHD of Dnmt3L, which in turn recruits the Dnmt3a, linking the H3K4 methylation state to DNA methylation (Fig. 3D) [Jia et al., 2007]. However, LSD1 also controls maintenance of DNA methylation by demethylating Dnmt1, as Dnmt1 methylation drastically decreases its stability [Wang et al., 2009]. Dnmt1 and/or Dnmt3 enzymes have been shown to interact directly with SNF2H, an ATPase subunit common to several chromatin remodeling complexes, the H3K9MTs Suv39h1, SetDB1 and G9a, components of the Polycomb repressive complex 2, heterochrmatin protein 1 (HP1), and HDACs [Fuks et al., 2000, 2001, 2003; Robertson et al., 2000; Geiman et al., 2004; Li et al., 2006; Vire et al., 2006; Epsztejn-Litman et al., 2008; reviewed in Cedar and Bergman, 2009]. While G9a and the PRC2 complex have been proposed to recruit Dnmts at their target genes, no functional hierarchy has been established in other cases. Nevertheless, the interaction network formed by Dnmts, MBPs, H3K9MTs, HP1, and HDACs (and including HP1 binding to H3K9MTs, methylated H3K9 and MeCP2) suggests the existence of positive feedback loop mechanisms stabilizing and possibly spreading silent chromatin states (Fig. 3E) [Lachner et al., 2001; Nielsen et al., 2002; Agarwal et al., 2007]. In addition, direct interaction between Dnmt1 and G9a at replication foci was proposed as a mechanism coupling maintenance of DNA and H3K9 methylation (Fig. 3B) [Esteve et al., 2006].

Finally, the remodeling factors of the SNF2H ATPase family Lsh and ATRX have been involved in the control of DNA methylation. ATRX mutations affect DNA methylation at rDNA loci and other repeats and ATRX interacts with HP1, MeCP2, and the PRC2 component Ezh2 [Gibbons et al., 2000; Nan et al., 2007]. Genetic targeting of Lsh resulted in global genomic hypomethylation and Lsh was shown to be required for de novo DNA methylation [Dennis et al., 2001; Zhu et al., 2006]. However, involvement of Lsh in chromatin remodeling has been questioned and Lsh was shown to mediate silencing of *Hox* loci by associating with both Dnmt3b and PRC1 [Xi et al., 2007; Myant and Stancheva, 2008].

DNA METHYLATION AND HIGHER ORDER CHROMATIN STRUCTURE

The formation of highly condensed pericentromeric heterochromatin domains (chromocenters) in mouse ESCs is clearly not affected by severe genomic hypomethylation and even near absence of DNA methylation [Tsumura et al., 2006; Gilbert et al., 2007]. However, there is still some discrepancy concerning the effect of hypomethylation on global levels of histone modifications. Severe genomic hypomethylation in ESCs was also reported to increase the clustering of chromocenters, whereas a modest increase in 5mC content at these domains, together with higher MBD proteins levels, resulted in increased clustering during differentiation of myoblasts to myotubes [Brero et al., 2005; Gilbert et al., 2007]. In addition, severe genomic hypomethylation was shown to restrict the mobility of linker histones H1 and H5 in ESCs [Gilbert et al., 2007]. Conversely, simultaneous genetic deletion of three histone H1 gene variants was reported to reduce methylation and alter the expression of some imprinted and X chromosome-linked genes, while leaving global DNA methylation patterns unaltered [Fan et al., 2005]. Thus, although DNA methylation has been shown to have some impact on higher order chromatin structure there is no clear consensus on the underlying mechanisms and direction of these effects.

CONCLUDING REMARKS

A major unresolved issue about the DNA methylation system (as well as other epigenetic pathways) concerns target specificity. Only few interactions between Dnmts and sequence-specific factors have been described and it cannot be excluded that most have gone undetected due to their sheer numbers and transient nature. Another possibility is that structural chromatin features, i.e., other epigenetic marks, generate a spectrum of affinity sites for Dnmt complexes. An example is demethylation of H3K4 by LSD1, which creates an affinity site for the PHD of Dnmt3L and thus may recruit the Dnmt3a-Dnmt3L complex. However, this only shifts the question of specificity to other epigenetic pathways. An exciting alternative is provided by small noncoding RNAs. While RNA-directed DNA methylation is well established in plants, a similar mechanism has only been recently described in mammalian cells for Piwi protein family-associated RNAs (piRNAs) involved in de novo methylation and silencing of transposable elements during differentiation of the male germ line [Kuramochi-Miyagawa et al., 2008]. However, the precise mechanism by which piRNAs direct de novo DNA methylation is not currently known. Also, changes in promoter methylation have been associated with small RNA-mediated transcriptional gene silencing in mammalian cells, but it is not clear whether these RNAs are actually guiding de novo methylation to the target sequence or methylation is a consequence of the silencing process [reviewed in Guil and Esteller, 2009].

Currently, complete epigenomes of a variety of different cell types are being established that include detailed information on genome wide DNA methylation, histone modifications, and nucleosome positioning as well as binding of regulatory factors and noncoding RNAs. In parallel, a rapidly growing number of factors, post-translational modifications and interactions are being identified that establish, maintain, and modify these epigenomes. The ultimate challenge for the next decades is to understand how these regulatory epigenetic networks change during development and disease and explain in quantitative terms their effect on gene expression patterns. Given the number of factors involved and the complexity of their interactions, it is clear that any comprehensive understanding of these epigenetic networks will require sophisticated and powerful bioinformatics tools.

ACKNOWLEDGMENTS

We apologize to colleagues whose work could not be cited due to space limitations. Work in the authors' laboratory is supported by Nanosystems Initiative Munich (NIM), BioImaging Network Munich (BIN) and grants from the Deutsche Forschungsgemeinschaft (DFG).

REFERENCES

Agarwal N, Hardt T, Brero A, Nowak D, Rothbauer U, Becker A, Leonhardt H, Cardoso MC. 2007. MeCP2 interacts with HP1 and modulates its heterochromatin association during myogenic differentiation. Nucleic Acids Res 35:5402–5408.

Arita K, Ariyoshi M, Tochio H, Nakamura Y, Shirakawa M. 2008. Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. Nature 455:818–821.

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.

Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V, Doderlein G, Maltry N, Wu W, Lyko F, Niehrs C. 2007. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445:671–675.

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

Bird A. 2007. Perceptions of epigenetics. Nature 447:396–398.

Bostick M, Kim JK, Esteve P-O, Clark A, Pradhan S, Jacobsen SE. 2007. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. Science 317:1760–1764.

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.

Brero A, Easwaran HP, Nowak D, Grunewald I, Cremer T, Leonhardt H, Cardoso MC. 2005. Methyl CpG-binding proteins induce large-scale chromatin reorganization during terminal differentiation. J Cell Biol 169: 733–743.

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

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.

Chuang LS-H, Ian H-I, Koh T-W, Ng H-H, Xu G, Li BFL. 1997. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. Science 277:1996–2000.

Citterio E, Papait R, Nicassio F, Vecchi M, Gomiero P, Mantovani R, Di Fiore PP, Bonapace IM. 2004. Np95 is a histone-binding protein endowed with ubiquitin ligase activity. Mol Cell Biol 24:2526–2535.

Datta J, Ghoshal K, Sharma SM, Tajima S, Jacob ST. 2003. Biochemical fractionation reveals association of DNA methyltransferase (Dnmt) 3b with Dnmt1 and that of Dnmt 3a with a histone H3 methyltransferase and Hdac1. J Cell Biochem 88:855–864.

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.

Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, Fuks F, Coco FL, 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.

Dodge JE, Okano M, Dick F, Tsujimoto N, Chen T, Wang S, Ueda Y, Dyson N, Li E. 2005. Inactivation of Dnmt3b in mouse embryonic fibroblasts results in DNA hypomethylation, chromosomal instability, and spontaneous immortalization. J Biol Chem 280:17986–17991.

Easwaran HP, Schermelleh L, Leonhardt H, Cardoso MC. 2004. Replication-independent chromatin loading of Dnmt1 during G2 and M phases. EMBO Rep 5:1181–1186.

Epsztejn-Litman S, Feldman N, Abu-Remaileh M, Shufaro Y, Gerson A, Ueda J, Deplus R, Fuks F, Shinkai Y, Cedar H, Bergman Y. 2008. De novo DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. Nat Struct Mol Biol 15:1176–1183.

Esteve PO, Chin HG, Pradhan S. 2005. Human maintenance DNA (cytosine-5)-methyltransferase and p53 modulate expression of p53-repressed promoters. Proc Natl Acad Sci USA 102:1000–1005.

Esteve PO, Chin HG, Smallwood A, Feehery GR, Gangisetty O, Karpf AR, Carey MF, Pradhan S. 2006. Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. Genes Dev 20: 3089–3103.

Fan Y, Nikitina T, Zhao J, Fleury TJ, Bhattacharyya R, Bouhassira EE, Stein A, Woodcock CL, Skoultchi AI. 2005. Histone H1 depletion in mammals alters global chromatin structure but causes specific changes in gene regulation. Cell 123:1199–1212.

Fatemi M, Hermann A, Pradhan S, Jeltsch A. 2001. The activity of the murine DNA methyltransferase Dnmt1 is controlled by interaction of the catalytic domain with the N-terminal part of the enzyme leading to an allosteric activation of the enzyme after binding to methylated DNA. J Mol Biol 309: 1189–1199.

Filion GJ, Zhenilo S, Salozhin S, Yamada D, Prokhortchouk E, Defossez PA. 2006. A family of human zinc finger proteins that bind methylated DNA and repress transcription. Mol Cell Biol 26:169–181.

Fouse SD, Shen Y, Pellegrini M, Cole S, Meissner A, Van Neste L, Jaenisch R, Fan G. 2008. Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. Cell Stem Cell 2:160–169.

Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T. 2000. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. Nat Genet 24:88–91.

Fuks F, Burgers WA, Godin N, Kasai M, Kouzarides T. 2001. Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription. EMBO J 20:2536–2544.

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

Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R. 2003. Induction of tumors in mice by genomic hypomethylation. Science 300:489–492.

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.

Georgel PT, Horowitz-Scherer RA, Adkins N, Woodcock CL, Wade PA, Hansen JC. 2003. Chromatin compaction by human MeCP2. Assembly of novel secondary chromatin structures in the absence of DNA methylation. J Biol Chem 278:32181–32188.

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.

Gilbert N, Thomson I, Boyle S, Allan J, Ramsahoye B, Bickmore WA. 2007. DNA methylation affects nuclear organization, histone modifications, and linker histone binding but not chromatin compaction. J Cell Biol 177:401–411.

Goll MG, Bestor TH. 2005. Eukaryotic cytosine methyltransferases. Annu Rev Biochem 74:481–514.

Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH. 2006. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science 311:395–398.

Gowher H, Leismann O, Jeltsch A. 2000. DNA of *Drosophila melanogaster* contains 5-methylcytosine. EMBO J 19:6918–6923.

Guil S, Esteller M. 2009. DNA methylomes, histone codes and miRNAs: Tying it all together. Int J Biochem Cell Biol 41:87–95.

Hashimoto H, Horton JR, Zhang X, Bostick M, Jacobsen SE, Cheng X. 2008. The SRA domain of UHRF1 flips 5-methylcytosine out of the DNA helix. Nature 455:826–829.

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.

Hendrich B, Tweedie S. 2003. The methyl-CpG binding domain and the evolving role of DNA methylation in animals. Trends Genet 19:269–277.

Hermann A, Gowher H, Jeltsch A. 2004. Biochemistry and biology of mammalian DNA methyltransferases. Cell Mol Life Sci 61:2571–2587.

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.

Johnson LM, Bostick M, Zhang X, Kraft E, Henderson I, Callis J, Jacobsen SE. 2007. The SRA methyl-cytosine-binding domain links DNA and histone methylation. Curr Biol 17:379–384.

Jorgensen HF, Ben-Porath I, Bird AP. 2004. Mbd1 is recruited to both methylated and nonmethylated CpGs via distinct DNA binding domains. Mol Cell Biol 24:3387–3395.

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.

Kim GD, Ni J, Kelesoglu N, Roberts RJ, Pradhan S. 2002. Co-operation and communication between the human maintenance and de novo DNA (cytosine-5) methyltransferases. EMBO J 21:4183–4195.

Kim JK, Esteve PO, Jacobsen SE, Pradhan S. 2009. UHRF1 binds G9a and participates in p21 transcriptional regulation in mammalian cells. Nucleic Acids Res 37:493–505.

Kunert N, Marhold J, Stanke J, Stach D, Lyko F. 2003. A Dnmt2-like protein mediates DNA methylation in Drosophila. Development 130:5083–5090.

Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Totoki Y, Toyoda A, Ikawa M, Asada N, Kojima K, Yamaguchi Y, Ijiri TW, Hata K, Li E, Matsuda Y, Kimura T, Okabe M, Sakaki Y, Sasaki H, Nakano T. 2008. DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. Genes Dev 22:908–917.

Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410:116–120.

Laird CD, Pleasant ND, Clark AD, Sneeden JL, Hassan KM, Manley NC, Vary JC Jr, Morgan T, Hansen RS, Stoger R. 2004. Hairpin-bisulfite PCR: Assessing epigenetic methylation patterns on complementary strands of individual DNA molecules. Proc Natl Acad Sci USA 101:204–209.

Lande-Diner L, Zhang J, Ben-Porath I, Amariglio N, Keshet I, Hecht M, Azuara V, Fisher AG, Rechavi G, Cedar H. 2007. Role of DNA methylation in stable gene repression. J Biol Chem 282:12194–12200.

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

Li H, Rauch T, Chen Z-X, Szabo PE, Riggs AD, Pfeifer GP. 2006. The histone methyltransferase SETDB1 and the DNA methyltransferase DNMT3A interact directly and localize to promoters silenced in cancer cells. J Biol Chem 281:19489–19500.

Liang G, Chan MF, Tomigahara Y, Tsai YC, Gonzales FA, Li E, Laird PW, Jones PA. 2002. Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. Mol Cell Biol 22:480–491.

Lorincz MC, Schubeler D, Hutchinson SR, Dickerson DR, Groudine M. 2002. DNA methylation density influences the stability of an epigenetic imprint and Dnmt3a/b-independent de novo methylation. Mol Cell Biol 22:7572–7580.

Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, Flavell RA, Lu B, Ming GL, Song H. 2009b. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science 323:1074–1077.

Margot JB, Aguirre-Arteta AM, Di Giacco BV, Pradhan S, Roberts RJ, Cardoso MC, Leonhardt H. 2000. Structure and function of the mouse DNA methyltransferase gene: Dnmt1 shows a tripartite structure. J Mol Biol 297:293–300.

Margot JB, Ehrenhofer-Murray AE, Leonhardt H. 2003. Interactions within the mammalian DNA methyltransferase family. BMC Mol Biol 4:7–15.

Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, Lander ES. 2008. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature 454:766–770.

Metivier R, Gallais R, Tiffoche C, Le Peron C, Jurkowska RZ, Carmouche RP, Ibberson D, Barath P, Demay F, Reid G, Benes V, Jeltsch A, Gannon F, Salbert G. 2008. Cyclical DNA methylation of a transcriptionally active promoter. Nature 452:45–50.

Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, Bibel M, Schubeler D. 2008. Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. Mol Cell 30:755–766.

Mund C, Musch T, Strodicke M, Assmann B, Li E, Lyko F. 2004. Comparative analysis of DNA methylation patterns in transgenic Drosophila overexpressing mouse DNA methyltransferases. Biochem J 378:763–768.

Myant K, Stancheva I. 2008. LSH cooperates with DNA methyltransferases to repress transcription. Mol Cell Biol 28:215–226.

Nan X, Hou J, Maclean A, Nasir J, Lafuente MJ, Shu X, Kriaucionis S, Bird A. 2007. Interaction between chromatin proteins MECP2 and ATRX is disrupted by mutations that cause inherited mental retardation. Proc Natl Acad Sci USA 104:2709–2714.

Nielsen PR, Nietlispach D, Mott HR, Callaghan J, Bannister A, Kouzarides T, Murzin AG, Murzina NV, Laue ED. 2002. Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. Nature 416:103–107.

Nikitina T, Shi X, Ghosh RP, Horowitz-Scherer RA, Hansen JC, Woodcock CL. 2007. Multiple modes of interaction between the methylated DNA binding protein MeCP2 and chromatin. Mol Cell Biol 27:864–877.

Okano M, Xie S, Li E. 1998. 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.

Papait R, Pistore C, Negri D, Pecoraro D, Cantarini L, Bonapace IM. 2007. Np95 is implicated in pericentromeric heterochromatin replication and in major satellite silencing. Mol Biol Cell 18:1098–1106.

Papait R, Pistore C, Grazini U, Babbio F, Cogliati S, Pecoraro D, Brino L, Morand AL, Dechampesme AM, Spada F, Leonhardt H, McBlane F, Oudet P, Bonapace IM. 2008. The PHD domain of Np95 (mUHRF1) is involved in large-scale reorganization of pericentromeric heterochromatin. Mol Biol Cell 19:3554–3563.

Rai K, Chidester S, Zavala CV, Manos EJ, James SR, Karpf AR, Jones DA, Cairns BR. 2007. Dnmt2 functions in the cytoplasm to promote liver, brain, and retina development in zebrafish. Genes Dev 21:261–266.

Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, Cairns BR. 2008. DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. Cell 135:1201–1212.

Robertson KD, Ait-Si-Ali S, Yokochi T, Wade PA, Jones PL, Wolffe AP. 2000. DNMT1 forms a complex with rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. Nat Genet 25:338–342.

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.

Schermelleh L, Haemmer A, Spada F, Rosing N, Meilinger D, Rothbauer U, Cristina Cardoso M, Leonhardt H. 2007. Dynamics of Dnmt1 interaction with the replication machinery and its role in postreplicative maintenance of DNA methylation. Nucl Acids Res 35:4301–43012.

Schmitz KM, Schmitt N, Hoffmann-Rohrer U, Schafer A, Grummt I, Mayer C. 2009. TAF12 recruits Gadd45a and the nucleotide excision repair complex to the promoter of rRNA genes leading to active DNA demethylation. Mol Cell 33:344–353.

Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA, Shinga J, Mizutani-Koseki Y, Toyoda T, Okamura K, Tajima S, Mitsuya K, Okano M, Koseki H. 2007. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature 450:908–912.

Silva AJ, Ward K, White R. 1993. Mosaic methylation in clonal tissue. Dev Biol 156:391–398.

Spada F, Haemmer A, Kuch D, Rothbauer U, Schermelleh L, Kremmer E, Carell T, Langst G, Leonhardt H. 2007. DNMT1 but not its interaction with the replication machinery is required for maintenance of DNA methylation in human cells. J Cell Biol 176:565–571.

Suzuki MM, Bird A. 2008. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet 9:465–476.

Tsumura A, Hayakawa T, Kumaki Y, Takebayashi S, Sakaue M, Matsuoka C, Shimotohno K, Ishikawa F, Li E, Ueda HR, Nakayama J, Okano M. 2006. Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. Genes Cells 11:805–814.

Uemura T, Kubo E, Kanari Y, Ikemura T, Tatsumi K, Muto M. 2000. Temporal and spatial localization of novel nuclear protein NP95 in mitotic and meiotic cells. Cell Struct Funct 25:149–159.

Unoki M, Nishidate T, Nakamura Y. 2004. ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. Oncogene 23: 7601–7610.

Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM, Bollen M, Esteller M, Di Croce L, de Launoit Y, Fuks F. 2006. The polycomb group protein EZH2 directly controls DNA methylation. Nature 439:871–874.

Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, Su H, Sun W, Chang H, Xu G, Gaudet F, Li E, Chen T. 2009. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet 41:125–129.

Xi S, Zhu H, Xu H, Schmidtmann A, Geiman TM, Muegge K. 2007. Lsh controls Hox gene silencing during development. Proc Natl Acad Sci USA 104:14366–14371.

Xu G-L, Bestor TH, Bourc'his D, Hsieh C-L, 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.

Yasui DH, Peddada S, Bieda MC, Vallero RO, Hogart A, Nagarajan RP, Thatcher KN, Farnham PJ, Lasalle JM. 2007. Integrated epigenomic analyses of neuronal MeCP2 reveal a role for long-range interaction with active genes. Proc Natl Acad Sci USA 104:19416–19421.

Yoon HG, Chan DW, Reynolds AB, Qin J, Wong J. 2003. N-CoR mediates DNA methylation-dependent repression through a methyl CpG binding protein Kaiso. Mol Cell 12:723–734.

Zhu H, Geiman TM, Xi S, Jiang Q, Schmidtmann A, Chen T, Li E, Muegge K. 2006. Lsh is involved in de novo methylation of DNA. EMBO J 25:335–345.