CTMI (2006) 301:21–44

Replication and Translation of Epigenetic Information

A. Brero · H. Leonhardt · M. C. Cardoso (✉)

Max Delbrück Center for Molecular Medicine, FVK, Wiltbergstr. 50,
13125 Berlin, Germany
cardoso@mdc-berlin.de

Abstract Most cells in multicellular organisms contain identical genetic information but differ in their epigenetic information. The latter is encoded at the molecular level by post-replicative methylation of certain DNA bases (in mammals 5-methyl cytosine at CpG sites) and multiple histone modifications in chromatin. In addition, higher-order chromatin structures are generated during differentiation, which might impact on genome expression and stability. The epigenetic information needs to be "translated" in order to define specific cell types with specific sets of active and inactive genes, collectively called the epigenome. Once established, the epigenome needs to be "replicated" at each cell division cycle, i.e., both genetic and epigenetic information have to be faithfully duplicated, which implies a tight coordination between the DNA replication machinery and epigenetic regulators. In this review, we focus on the molecules and mechanisms responsible for the replication and translation of DNA methylation in mammals as one of the central epigenetic marks.

1 Introduction

The term "post-genomic era," which is often used to classify the present scientific period, does not only stress the fact that the scientific community has finally reached beyond the mere deciphering of genomes, it also indicates that there is another level of genomic information apart from the one-dimensional nucleotide sequence. This *epi* (above/outside) genetic information is respon-

sible for defining a cell type-specific state of the genome with a distinct set of active and inactive genes, the so-called epigenome. While the genome in a multicellular organism is identical for all cell types (with minor exceptions), the epigenome is potentially dynamic and cell type specific.

Epigenetic mechanisms have been reported to act by very different means, and an exhaustive description of the phenomenon is far from being completed. Some of these mechanisms act at the chromatin level as the methylation of DNA or the modification of histones by various functional groups including methyl, acetyl, phosphate, ADP-ribosyl groups or even such small proteins as ubiquitin or SUMO (reviewed, e.g., in Felsenfeld and Groudine 2003). Other epigenetic modifications of chromatin include histone variants as well as chromatin-associated proteins like Polycomb group proteins. A different kind of epigenetic mechanism has been proposed to act at a global, topological scale, through the specific position of genes within the nucleus relative to functional nuclear subcompartments such as nucleoli, heterochromatin, splicing compartments, etc. (reviewed, e.g., in: Cremer and Cremer 2001; Fisher and Merkenschlager 2002; Spector 2003). An emerging view is that the different epigenetic mechanisms can feedback onto each other, either strengthening a specific epigenetic state or weakening it, thereby enabling transition between transcriptionally permissive and repressive states of genes. In the present review we will address the propagation and translation of epigenetic information with the focus on DNA methylation in mammals.

2 DNA Methylation

The modification of nucleotides in the DNA by covalently bound methyl groups was already described in the late 1940s and early 1950s (Hotchkiss 1948; Wyatt 1951). In the 1960s it was proposed that DNA methylation might be involved in a protection mechanism (1) against the integration of foreign DNA or (2) in rendering host DNA resistant to DNAses directed against foreign DNA (Srinivasan and Borek 1964). The latter idea went hand in hand with the discovery of bacterial restriction enzymes, which were thought to protect methylated bacterial host DNA from "invading" bacterial and viral DNA by specific digestion of the unmodified "parasitic" DNA (reviewed in Arber and Linn 1969). It was not before 1975, though, that methylation of DNA in mammals was suggested to be connected with transcriptional regulation (Holliday and Pugh 1975; Riggs 1975).

DNA methylation is found in many different organisms including prokaryotes, fungi, plants, and animals, where it can serve different functions. Methyl

groups in the DNA are found at the C^5 position of cytosines giving rise to 5-methyl cytosine (5mC) or at N^6 position of adenines resulting in N^6-methyladenine (6mA). As already noted, methylation of DNA in bacteria is involved in a protection mechanism in which restriction endonucleases specifically digest foreign DNA by discriminating unmodified invader DNA sequences from methylated host DNA. In eukaryotic cells, the majority of methylated bases are cytosines, with only some, mostly unicellular organisms, showing low levels of methylated adenines (Gorovsky et al. 1973; Cummings et al. 1974; Hattman et al. 1978). Methylation levels of eukaryotic DNA vary widely, from undetectable as in budding/fission yeast, nematodes or in adult *Drosophila melanogaster* flies over intermediate levels in mammals (2–8 mol%) up to high levels, reaching approximately 50 mol% in higher plants (see Doerfler 1983). In humans, approximately 1% of all DNA bases are estimated to be 5mC (Kriaucionis and Bird 2003). The sequence context in which methylated bases are found in eukaryotes is also variable. In mammals, for example, methylation is mainly found in CpG dinucleotides, with this "mini"-palindrome methylated on both strands. In fact 60%–90% of CpGs are methylated in mammalian genomes with the exception of so-called CpG islands, which are stretches of roughly 1 kb that frequently coincide with promoter regions. These sequences, which are thought to be involved in transcriptional regulation, comprise roughly 1% of the mammalian genome. Exceptions to the rule that CpG islands are generally unmethylated are silenced genes on the inactive X-chromosome and at imprinted loci, where, depending on the parental origin, one allele is silenced. In contrast to mammals, methylation in fungi (reviewed in Selker 1997) and in plants (reviewed in Tariq and Paszkowski 2004) is not limited to CpG sites, with also CpNpG sequences being frequently methylated.

From an evolutionary point of view, DNA methylation is thought to represent an ancient mechanism, as the catalytic domain of DNA methyltransferases (Dnmts), the enzymes responsible for adding methyl groups to DNA, appears to be conserved from prokaryotes to humans (Kumar et al. 1994). However, in the course of genome evolution there must have been adaptations concerning how methyl marks were eventually utilized, since in different taxa DNA methylation appears to be involved in different functions. While in prokaryotes and fungi methylation appears mainly to serve protection needs of the host genome, in higher eukaryotes transcriptional silencing seems to be the main, though not the only, purpose. A major change concerning the genomic organization as well as the extent of DNA methylation is thought to have occurred at the origin of vertebrate evolution, where DNA methylation seems to have changed from a fractional organization, to a global one (Tweedie et al. 1997). In non-vertebrates, methylated DNA does not neces-

sarily correlate with transposable elements or other functional chromosomal regions and appears not to be involved in transcriptional regulation, as no correlation could be found between transcription and methylation, neither for housekeeping genes, nor for tissue-specific genes (Tweedie et al. 1997). In contrast, in mammals DNA methylation is implicated in many different aspects of transcriptional control including developmentally regulated genes, imprinted genes, and genes affected by X-inactivation. Nevertheless, it is also crucial for preventing spreading of potentially "parasitic" DNA elements like transposable sequences, thereby ensuring genome stability. Defects in DNA methylation have been shown to be involved in several pathological situations including cancer and other diseases such as Rett syndrome (RTT) or immunodeficiency, centromere instability, facial anomalies (ICF) syndrome.

In the following sections, we will review two important aspects of DNA methylation, with an emphasis on the situation in mammals. In the first part we will reason how methylation marks are maintained in proliferating cells, i.e., how they are *replicated*, while in the second part we will concentrate on the question of how methylated CpGs are functionally interpreted in terms of transcriptional regulation, i.e., how the methyl cytosine information is *translated*.

3 Replication of DNA Methylation

DNA methylation represents a post-synthetic modification, i.e., nucleotides are modified *after* they have been incorporated into the DNA. With respect to their substrate preference, two different kinds of Dnmts are distinguished: (1) de novo Dnmts, which add methyl groups to completely unmethylated DNA and (2) maintenance Dnmts that show a higher affinity for hemimethylated DNA, i.e., DNA where only one strand of the CpG palindrome is modified. Hemimethylated DNA results from the replication of methylated regions. In both cases, the methyl-group donor is *S*-adenosyl-L-methionine (SAM). The three main, catalytically active Dnmts in mammals are Dnmt1, which is thought to serve as maintenance methyltransferase and Dnmt3a and 3b as de novo methylating enzymes. A summary of the mouse Dnmt protein family and their domains is shown in Fig. 1.

Dnmt2 is expressed ubiquitously at low levels, but although it is among the most highly conserved Dnmts among different species all the way down to fission yeast, in most organisms it could not yet be shown to possess catalytic activity (Okano et al. 1998; Yoder and Bestor 1998; discussed in Robertson 2002). In *D. melanogaster,* however, Dnmt2 is responsible for the low level

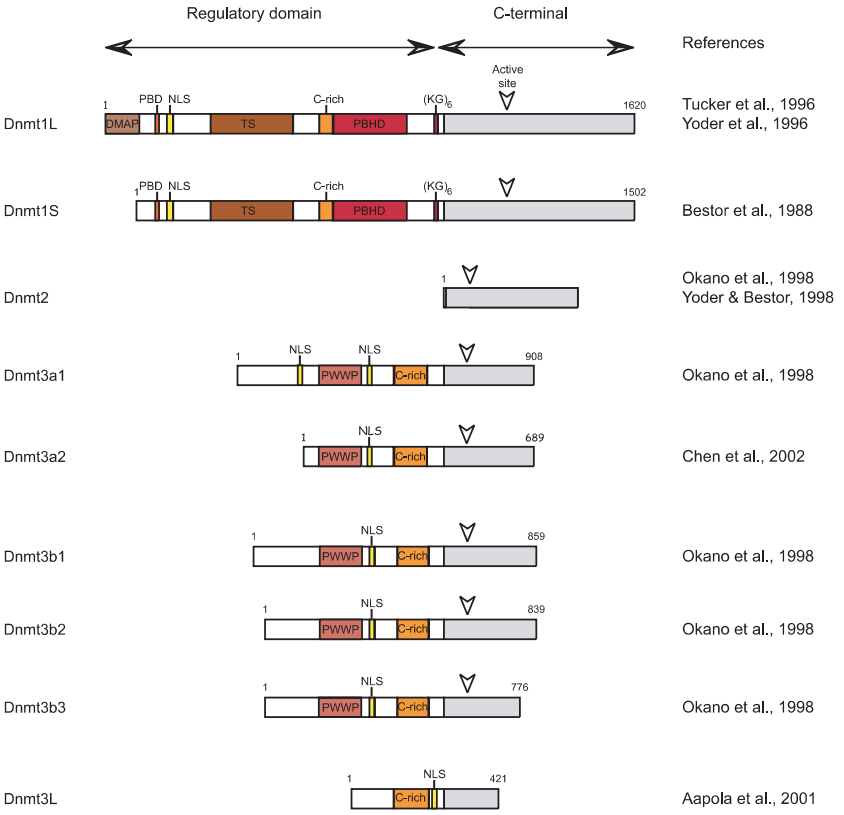

Fig. 1 Organization of the mouse Dnmt protein family. *Numbers* represent amino acid positions. *C-rich*, Cys-rich sequence; *DMAP*, DMAP1 binding domain; *$(KG)_6$*, Lys-Gly repeat; *NLS*, nuclear localization signal; *PBD*, PCNA binding domain; *PBHD*, Polybromo1 homology domain; *PWWP*, Pro-Trp-Trp-Pro domain; *TS*, targeting sequence

DNA methylation found during embryonic stages (Kunert et al. 2003). Due to its evolutionary conservation, Dnmt2 might well represent the ancestral Dnmt protein.

The de novo methylating enzymes Dnmt3a and 3b are supposed to be responsible for methylation of the embryonic genome after implantation, i.e., after the parental genomes have been demethylated (Okano et al. 1999). Dnmt3a and Dnmt3b have been shown to be catalytically active in vitro as well as in vivo, and transcripts were found in embryonic stem (ES) cells, in the early embryo as well as in adult tissue and in tumor cells (see citations in Robertson et al. 1999). Two isoforms of Dnmt3a were described, one reported

to bind euchromatin and the other heterochromatin (Okano et al. 1998; Chen et al. 2002). *Dnmt3a* knockout ES cell lines appeared to be normal concerning their de novo methylation potential, and null mice developed inconspicuously until birth, but shortly after showed decreased growth and died by 4 weeks of age (Okano et al. 1999). Dnmt3b shows at its N-terminus only little sequence homology to Dnmt3a, and unlike Dnmt3a, its expression is low in most tissues, but high in testis, so that an implication in methylation during spermatogenesis has been proposed (Okano et al. 1998; Robertson et al. 1999; Xie et al. 1999). Its localization in centromeric regions in ES cells (Bachman et al. 2001) and the observation that mutant *Dnmt3b*$^{-/-}$ cells exhibit a decreased methylation of minor satellite repeats (Okano et al. 1999) suggested a role in centromeric satellite methylation. Dnmt3b appears to be more important during embryonic development than Dnmt3a, since no viable null mice were obtained (Okano et al. 1999). Mutations in *Dnmt3b* in humans cause so-called ICF syndrome, where pericentric repeats are hypomethylated (Hansen et al. 1999; Okano et al. 1999; Xu et al. 1999). Several Dnmt3b splicing isoforms have been found. The eight variants described in mouse and the five in humans are expressed in a tissue-specific manner, yet not all of them appear to be catalytically active. Figure 1 lists only the three first-described Dnmt3b isoforms.

Within the Dnmt3 family but more distantly related is the Dnmt3L protein that lacks the conserved motifs of C5-methyltransferases and was found to be highly expressed in mouse embryos and testis (Aapola et al. 2001). *Dnmt3L* null mice show methylation defects at maternal imprints (Bourc'his et al. 2001) but otherwise a normal genome-wide methylation pattern, which suggests that Dnmt3L is involved in the establishment of maternal imprints, probably by recruiting Dnmt3a or 3b to target loci, either directly or indirectly.

The Dnmt1 enzyme was the first to be cloned (Bestor et al. 1988) and was shown to be essential for development, since null mice die at mid-gestation (Li et al. 1992). Interestingly *Dnmt*1$^{-/-}$ ES cells are viable and show normal morphology and a 5mC level that is still 30% of that in wild-type cells, suggesting some compensatory methylation activity (Li et al. 1992), likely due to Dnmt3a/3b enzymes. Across various mammalian species, the N-terminus of Dnmt1 appears to be rather variable, while the catalytic C-terminus is more conserved (Margot et al. 2000). The intracellular distribution of Dnmt1 is rather dynamic throughout the cell cycle. The enzyme is diffusely distributed throughout the nucleoplasm during most of G1, associates with subnuclear sites of DNA replication during S-phase (Leonhardt et al. 1992), and binds to chromatin, with preference to pericentric heterochromatin, during G2 and M-phases (Easwaran et al. 2004). This complex cell cycle distribution of Dnmt1 has also been exploited to construct cell-cycle marker systems (Easwaran et

al. 2005). Since Dnmt1 messenger (m)RNA has also been found in low proliferative tissue (Robertson et al. 1999), where only few cells are suspected to be actually replicating DNA, it has been proposed that Dnmt1 might exert an additional function beyond methylating hemimethylated DNA during S-phase. In fact, isoforms of Dnmt1 have been found that could account for additional functions. The originally cloned Dnmt1 (Bestor et al. 1988) was found later to be missing a 118 amino acid sequence at its N-terminus (Tucker et al. 1996; Yoder et al. 1996). This longer Dnmt1 protein (Dnmt1L; 1,620 amino acids) is expressed in most proliferating somatic cells, while the original shorter Dnmt1 protein (Dnmt1S; 1,502 amino acids) accumulates specifically during oocyte growth (Mertineit et al. 1998). While at the protein level, two forms are known, at the mRNA level, three isoforms with differing first exons/promoters have been described. In addition to the predominant somatic isoform, two sex-specific isoforms were isolated. One isoform is the only one expressed in oocytes and corresponds at the protein level to the shorter form (Mertineit et al. 1998). It localizes in the cytoplasm of mature oocytes, except for the 8-cell stage, where it is transiently relocated into the nucleus (Carlson et al. 1992; Cardoso and Leonhardt 1999). Since knockout female but not male mice were infertile, with embryos from deficient females showing defective methylation pattern at imprinted loci, the current idea is that oocyte Dnmt1 and especially its nuclear localization at the 8-cell stage is important for maintaining imprints (Howell et al. 2001). During mouse preimplantation development, while the genome is globally demethylated, this Dnmt1 form appears to be responsible for keeping the retrotransposable element IAP (intracisternal A-type particle) methylated and thus silent (Gaudet et al. 2004). Silencing of such mobile elements is thought to be crucial to prevent transcriptional activation and potential mutagenesis by transposition. The second sex-specific isoform was originally detected in pachytene spermatocytes (Mertineit et al. 1998). The same isoform, however, was found also in differentiated myotubes, instead of the ubiquitously expressed Dnmt1, which is downregulated upon differentiation (Aguirre-Arteta et al. 2000). Since myotube nuclei show no DNA replication, this isoform might serve a function that is independent of DNA synthesis. Both oocyte and spermatocyte/skeletal muscle mRNA isoforms give rise to the shorter Dnmt1 protein form.

The marked preference of Dnmt1 for hemimethylated DNA together with its specific association with replication machinery during S-phase via binding to proliferating-cell nuclear antigen (PCNA) (Leonhardt et al. 1992; Chuang et al. 1997; Easwaran et al. 2004) make it a strong candidate for mediating the propagation of the DNA methylation pattern at each cell division cycle. As shown in Fig. 2, during replication of DNA, the hemi-methylated CpG sites in the newly synthesized strand are post-replicatively modified by the activity

of Dnmt1. Since Dnmt1 is a catalytically slow enzyme (Pradhan et al. 1997), its prolonged association in G2 and M-phases with chromatin could allow sufficient time for full methylation of all hemimethylated sites, in particular at heavily methylated heterochromatic sequences (Easwaran et al. 2004). In addition, Dnmt1 has been reported to interact with histone deacetylases (HDACs) (Fuks et al. 2000; Robertson et al. 2000; Rountree et al. 2000) and might serve as a loading platform for these chromatin modifiers. Concomitantly, methyl-CpG-binding domain (MBD) proteins, recognizing the newly generated modified CpGs, have been also shown to recruit HDACs (Jones et al. 1998; Nan et al. 1998; Ng et al. 1999) and can thereby further contribute to the replication of the histone modifications upon DNA replication. In this regard, there is increasing evidence of crosstalk between histone modifications and DNA methylation. In parallel to these mechanisms for replication of epigenetic information, the random distribution of "old" histones between the two replicated DNA strands implies that modifications such as histone methylation are passed onto the nucleosomes assembled at the newly replicated strands. Factors such as HP1, which recognizes specific methylation forms of histone H3 (Lachner et al. 2001), can then bind the replicated chromatin, recruit histone methyltransferases (HMTs) (Lehnertz et al. 2003) and "spread" the histone methylation marks onto the adjacent, previously deacetylated histones.

Although many enzymes have been described that can actually add methyl groups to the DNA, much less is known about DNA demethylases. The existence of such enzymes, however, is almost certain, since active demethylation of the paternal genome during preimplantation development has been evidenced (Mayer et al. 2000). Similarly, there must be demethylases, which can remove imprints in the course of germ cell development, in order to set the novel parental identity. Candidate enzymes for DNA demethylation include, on the one hand, glycosylases, which in effect resemble a "base excision DNA repair activity" where the methylated cytosines are removed, resulting in an abasic site and single strand breaks that have to be consecutively repaired (Jost et al. 2001; Vairapandi 2004). Another proposed mechanism includes direct demethylation of 5mC, via the methylated CpG binding protein MBD2 (Bhattacharya et al. 1999). Since MBD2 has also been reported to be involved in 5mC-dependent transcriptional repression (Hendrich and Tweedie 2003) (see following section), it was proposed that it might exert a dual, promoter-specific role as a repressor through binding of 5mC and as an activator through active DNA demethylation (Detich et al. 2002). However, the demethylating activity of MBD2 could not yet be reproduced and is hence disputed (Vairapandi 2004).

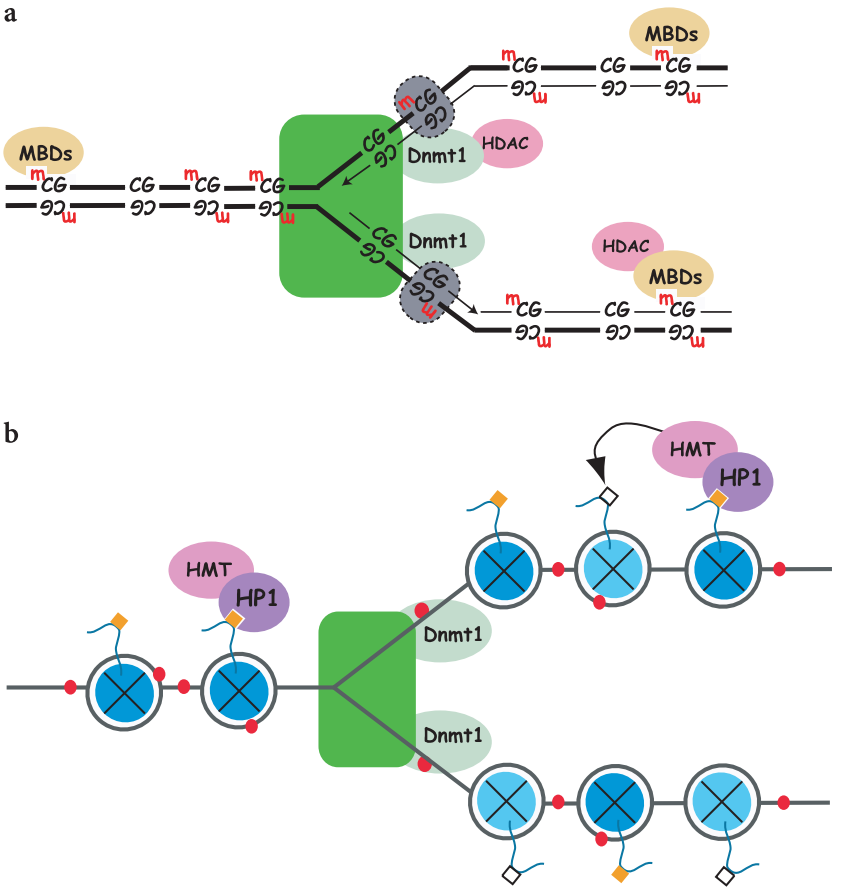

Fig. 2a, b Replication of epigenetic information. **a** A replication fork is shown where Dnmt1 associated with the replication machinery (*green box*) is copying the methylation mark (*m*) at hemimethylated CpG sites, which are then recognized and bound by methyl-CpG-binding domain (MBD) proteins. Both MBD proteins and Dnmt1 recruit histone deacetylases (HDACs), thereby maintaining the deacetylated chromatin state. **b** The same replication fork is shown from a nucleosomal view. Nucleosomes are shown as *blue circles*, with methylated histone H3 tails as *filled yellow squares* and 5mC as *red dots*. Histones bearing repressive methylated lysine residues are distributed randomly onto replicated daughter strands. Binding of HP1 to methylated histones can recruit histone methyltransferase (HMT) that modify lysine residues of the newly incorporated histones (*light blue circles*)

4
Translation of DNA Methylation

The precise mode of action of how DNA methylation modulates transcription is far from being understood. In fact, different mechanisms could account for controlling gene expression at different loci. Though DNA methylation in general is associated with transcriptional silencing, in some cases methylation has been shown to induce expression. This has been demonstrated for the imprinted *Igf2* locus, where methylation of a differentially methylated region (DMR) on the maternal chromosome prevents binding of CTCF (CCCTC-binding factor), which results in a positive enhancer function (Bell and Felsenfeld 2000; Hark et al. 2000; Kanduri et al. 2000; Szabo et al. 2000). Transcriptional silencing mediated by methylation of CpGs near promoter regions is thought to occur by at least two different mechanisms. One possibility is that methylation of specific target sites simply abolishes binding of transcription factors or transcriptional activators by sterical hindrance. Another increasingly important mechanism involves the specific recognition and binding of factors to methylated DNA, triggering different kinds of downstream responses, entailing (or not) further chromatin modifications. In mammals, there are several known methyl-CpG-binding proteins. The MBD protein family members share a conserved *m*ethyl-CpG-*b*inding *d*omain (MBD) (Hendrich and Bird 1998). While MeCP2, MBD1, and MBD2 have been shown to act as transcriptional repressors, MBD4 appears to be involved in reducing the mutational risk from potential C→T transitions, which result from deamination of 5mC. A fifth member of the MBD family, MBD3 does not bind to methylated DNA (Hendrich and Tweedie 2003), but is a constituent of the NuRD (nucleosome remodeling and histone deacetylation) corepressor complex. A further, recently detected 5mC-binding protein is Kaiso, which shows no sequence conservation with MBD proteins but also functions as a transcriptional repressor (Prokhortchouk et al. 2001). In contrast to MBDs, Kaiso appears to bind via a zinc-finger motif in a sequence-specific manner at sequences containing two symmetrically methylated CpGs. A recent study in *Xenopus* revealed an essential role of Kaiso as a methylation-dependent global transcriptional repressor during early development (Ruzov et al. 2004).

In mammals, the MBD family comprises five members: MBD1–4 and MeCP2. All of them except MBD3 share a functional MBD that is responsible for targeting the proteins to 5mC sites. In mouse cells this can be readily seen by the increased concentration of MBD proteins at pericentric heterochromatin, which is highly enriched in 5mC (Lewis et al. 1992; Hendrich and Bird 1998). A summary of the mouse MBD protein family and their domains is shown in Fig. 3.

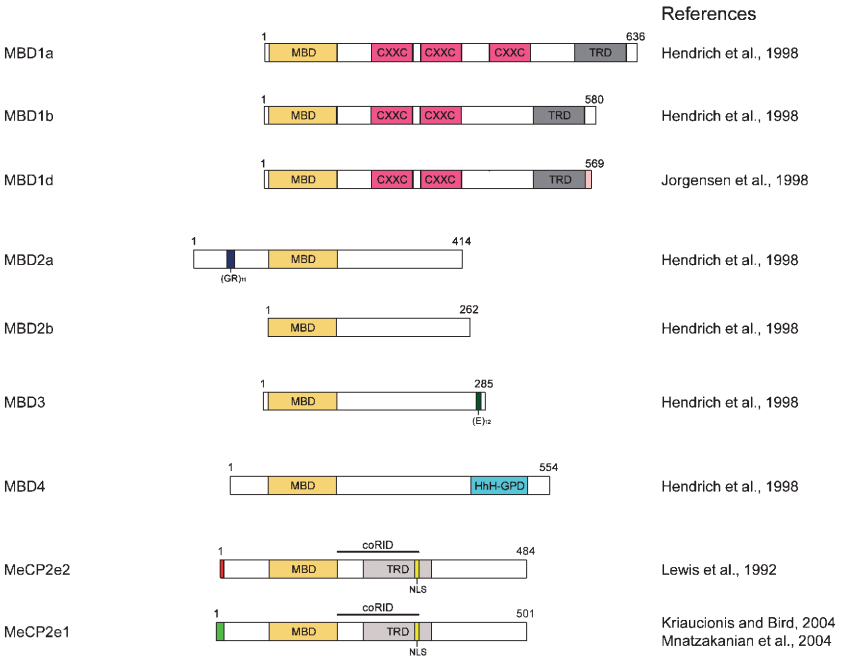

Fig. 3 Organization of the mouse MBD protein family. *Numbers* represent amino acid positions. *coRID*, corepressor interacting domain; *CXXC*, Cys-rich domain; $(E)_{12}$, Glu repeat; $(GR)_{11}$, Gly-Arg repeat; *MBD*, methyl-CpG-binding domain; *HhH-GPD*, DNA N-Glycosylase domain; *TRD*, transcriptional repressor domain

MBD2 and 3 show a high conservation, sharing the same genomic structure except for their intron length (Hendrich et al. 1999a). Since homologous expressed sequence tags (ESTs) for MBD2/3 were also found in invertebrates, it is thought to represent the ancestral protein from which all other family members have been derived (Hendrich and Tweedie 2003). The increase in number of 5mC binding proteins from invertebrates to vertebrates is believed to have paralleled the increase in DNA methylation (see Sect. 2, "DNA Methylation"), as this would have enabled a fine-tuning of methylation-dependent silencing on the one hand, as well as lowered the mutational risks emerging from spontaneous deamination on the other (Hendrich and Tweedie 2003).

In mammals, MBD3 does not bind to methylated CpGs due to two amino acid substitutions within the MBD (Saito and Ishikawa 2002). Other vertebrates, however, such as frogs, have two MBD3 forms, one of which retains a 5mC-binding ability (Wade et al. 1999). Sequence homology predicts a similar situation for the pufferfish and the zebrafish (Hendrich and Tweedie 2003).

MBD3 in mammals is a constituent of the NuRD corepressor complex. NuRD is found in many organisms including plants and plays an important role in transcriptional silencing via histone deacetylation. Though MBD3 has been shown to be essential for embryonic development (Hendrich et al. 2001), its function within the NuRD multiprotein complex has still to be clarified. MBD2 interacts with the NuRD complex making up the MeCP1 complex (methyl-CpG-binding protein), which was actually the first methyl-CpG-binding activity isolated in mammals (Meehan et al. 1989). In spite of the many potential binding sites of MBD2, it does not appear to act as a global transcriptional repressor. In fact, only one target gene of MBD2 has been described until now, and that is *Il4* during mouse T cell differentiation (Hutchins et al. 2002). Here loss of MBD2 has been shown to correlate with a leaky instead of a complete repression. Consequently, it has been hypothesized that MBD2 might rather act in "fine-tuning" transcriptional control by reducing transcriptional noise at genes, which are already shut off (Hendrich and Tweedie 2003). Alternatively, the lack of a global de-repression of methylated genes upon MBD2 loss could be explained by redundancy among MBD family members. Studies abrogating several MBD proteins at the same time will help to answer this question. An interesting phenotype of MBD2$^{-/-}$ mice is that affected female animals neglect their offspring due to an unknown neurological effect (Hendrich et al. 2001). MBD2b is an isoform that is generated by using an alternative translation start codon generating a protein that lacks 140 N-terminal amino acids (Hendrich and Bird 1998). Surprisingly, it has been reported to possess a demethylase activity (see previous section and Bhattacharya et al. 1999). In gene reporter assays, it was even shown to act as a transcriptional activator (Detich et al. 2002). Thus, it has been proposed that MBD2 could act as both a transcriptional repressor and stimulator. It should be added, though, that other groups have not been able to reproduce the demethylase activity of MBD2b, so the existence of this activity is still controversial (discussed in Wade 2001).

MBD1 is exceptional among the transcriptionally repressive MBDs, since it can suppress transcription from both methylated and unmethylated promoters in transient transfection assays (Fujita et al. 1999). Four splicing isoforms have been described in humans (Fujita et al. 1999) and three in mouse (Jorgensen et al. 2004), with the major difference being the presence of three versus two CXXC cysteine-rich regions (see Fig. 3). The presence of the most C-terminal CXXC motifs in mouse was shown to be responsible for its binding to unmethylated sites (Jorgensen et al. 2004) and for its capacity to silence unmethylated reporter constructs (Fujita et al. 1999). The repression potential of MBD1 seems to rely on the recruitment of HDACs, although, most probably, different ones from those engaged in MBD2 (and MeCP2) silencing (Ng et

al. 2000). Similar to MBD2, MBD1$^{-/-}$ mice exhibit neurological deficiencies, as they show reduced neuronal differentiation and have defects in spatial learning as well as in hippocampus long-term potentiation (Zhao et al. 2003).

MBD4 is the only member within the MBD family that is not involved in transcriptional regulation. Instead, it appears to be implicated in reducing the mutational risk that is imminent in genomes with high methylation levels, by transitions of 5mC→T via deamination. This transition poses a bigger problem for the DNA repair machinery than C→U transitions, which result from the deamination of unmethylated cytosines, since the former results in G–T mismatches, in which the mismatched base (G or T) cannot readily be identified. In contrast, uracil in G–U mismatches can easily be pinpointed as the "wrong" base, since it is not a constituent of DNA. Accordingly, MBD4 possesses a C-terminal glycosylase moiety that can specifically remove Ts from G–T mismatches (Hendrich et al. 1999b; see Fig. 3). In fact, its preferred binding substrate is 5mCpG/TpG, i.e., the deamination product of the 5mCpG/5mCpG dinucleotide. Indeed mutation frequency analysis in MBD4$^{-/-}$ mice revealed an approximately threefold increase in C→T transitions at CpGs compared to wild-type cells (Millar et al. 2002; Wong et al. 2002), which supports the idea of MBD4 being a mutation attenuator.

Since MeCP2 was the first methyl-CpG-binding protein to be cloned and the second methylated DNA binding activity to be isolated after MeCP1, it is often referred to as the founding member of the MBD family. A single methylated CpG dinucleotide has been shown to be sufficient for binding (Lewis et al. 1992). In transient transfection assays with methylated gene reporter in *Xenopus* and in mice it was demonstrated that MeCP2 functions as a transcriptional repressor, at least in part via interaction with the Sin3 corepressor complex, which contains histone deacetylases 1 and 2 (Jones et al. 1998; Nan et al. 1998). An approximately 100-amino-acid-containing transcriptional repression domain (TRD) in the middle of the protein has been shown to be critical for transcriptional silencing (Nan et al. 1997). Apart from the recruitment of HDACs, MeCP2 has been shown to associate with a histone methyltransferase activity specifically modifying histone H3 at lysine 9, which is known to represent a transcriptionally repressive chromatin label (Fuks et al. 2003). In addition, MeCP2 has recently been found to interact with components of the SWI/SNF-related chromatin-remodeling complex, suggesting a novel potential MeCP2-dependent silencing mechanism (Harikrishnan et al. 2005). Moreover, MeCP2 can induce compaction of oligonucleosomes in vitro, which could additionally suppress transcription in vivo through a dense chromatin conformation that is incompatible with the binding of factors relevant for transcriptional activation (Georgel et al. 2003). In summary, MeCP2 could translate the DNA methylation mark directly by preventing the access

of transcriptional activators to promoters/enhancers or indirectly by either recruiting modifiers of histones such as histone deacetylases (see also Fig. 2) and methyltransferases or by compacting chromatin.

With the idea in mind that MeCP2 might act as a global transcription repressor, it was very surprising that an expression profiling analysis comparing MeCP2 null mice with normal animals revealed only subtle changes in the mRNA profiles of brain tissues (Tudor et al. 2002). This apparent lack of global de-repression in the absence of MeCP2 resembles a similar situation as described for MBD2$^{-/-}$ mice (as discussed earlier in this section). Possible reasons for this observation could be either that other MBD proteins can compensate for the loss of MeCP2, or that the changes in transcription levels induced by MeCP2 deficiency are so small that they are undetectable with current microarray technology. This supports the rationale that MBDs might act as reducers of transcriptional noise rather than to shut down active genes (Hendrich and Tweedie 2003). On the other hand, it could well be that MeCP2 represses genes in a tissue- and/or time-specific fashion. Matarazzo and Ronnett, for example, using a proteomic approach, found substantial differences in protein levels between MeCP2-deficient and wild-type mice (Matarazzo and Ronnett 2004). Importantly, they showed that the degree of differences varied depending on the analyzed tissue (olfactory epithelium vs olfactory bulb) and the age of the animals (2 vs 4 weeks after birth). Apart from a potential global effect, MeCP2 has recently been linked to the regulation of two specific target genes. The genes of Hairy2a in *Xenopus* (Stancheva et al. 2003) and brain-derived neurotropic factor (BDNF) in rat (Chen et al. 2003) and mice (Martinowich et al. 2003)—both are proteins involved in neuronal development and differentiation—have methylated promoters with bound MeCP2, which is released upon transcriptional activation. Recently MeCP2 was shown to be involved in the transcriptional silencing of the imprinted gene *Dlx5* via the formation of a chromatin loop structure (Horike et al. 2005).

MeCP2 is expressed ubiquitously in many tissues of humans, rats, and mice, although at variable levels. Several lines of evidence argue that MeCP2 expression increases during neuronal maturation and differentiation (Shahbazian et al. 2002b; Jung et al. 2003; Balmer et al. 2003; Cohen et al. 2003; Mullaney et al. 2004). In a recent study, it was shown that MeCP2 and MBD2 protein levels increase also during mouse myogenesis along with an increase in DNA methylation at pericentric heterochromatin (Brero et al. 2005). Moreover, it was demonstrated that MeCP2 and MBD2 are responsible for a major reorganization of pericentric heterochromatin during terminal differentiation that leads to the formation of large heterochromatic clusters (Brero et al. 2005). This finding provides the link between a protein(s) (MeCP2/MBD2) and chromatin organization and assigns it a direct role in changes of the

3D chromatin topology during differentiation. The latter represents yet another level of epigenetic information beyond the molecular composition of chromatin.

In agreement with its substrate specificity, MeCP2 localizes mainly at heavily methylated DNA regions. In mouse nuclei, for example, MeCP2 intensely decorates pericentric heterochromatin (Lewis et al. 1992). In human cells, however, the intranuclear distribution of MeCP2 was found to deviate from the pattern in mouse, in that it did not strictly colocalize with methylated DNA, pericentric satellite sequences, or heterochromatic regions [visualized by intense 4′-6′-diamidino-2-phenylindole (DAPI) staining; Koch and Stratling 2004]. Intriguingly, the authors found an additional binding affinity of MeCP2 for TpG dinucleotides and proposed a sequence-specific binding defined by adjacent sequences. By using an immunoprecipitation approach, they revealed an association of MeCP2 with retrotransposable elements, especially with Alu sequences, and with putative matrix attachment regions (MARs). In this respect, it should be added that the MeCP2 homolog in chicken (named ARBP) was originally isolated as a MAR binding activity (von Kries et al. 1991), even before rat MeCP2 was actually described for the first time (Lewis et al. 1992), yet its homology to the rat protein was noticed only later (Weitzel et al. 1997). Interestingly, ARBP/MeCP2 binding in chicken appears not to be dependent on CpG methylation (Weitzel et al. 1997). Since the results in human cells were obtained using a breast cancer cell line (MCF7), it will be interesting to investigate further human cell types, including primary cells, to further clarify MeCP2 binding specificity in human cells.

Two studies have lately reported a second MeCP2 splicing isoform, which yields a protein with a slightly different N-terminal end, due to the utilization of an alternative translation start codon (Kriaucionis and Bird 2004; Mnatzakanian et al. 2004; Fig. 3). Surprisingly this new MeCP2 mRNA appears to be much more abundant in different mouse and human tissues than the originally described isoform. Fluorescently tagged fusions of both proteins, though, show the same subnuclear distribution in cultured mouse cells (Kriaucionis and Bird 2004). An antibody raised against the "old" isoform was shown to recognize also the novel variant (Kriaucionis and Bird 2004). Consequently, in previous immunocytochemical studies most probably both isoforms have been detected. The differences between both isoforms are only subtle, with the new protein having a 12 (human) and 17 (mouse) amino acid longer N-terminus followed by a divergent stretch of 9 amino acids. Since neither the MBD nor the TRD are affected by the changes, both proteins are anticipated to be functionally equivalent.

As already noted, MeCP2 expression appears to be correlated with differentiation and development. Its implication in neuronal differentiation is

further supported by its involvement in a human neurodevelopmental disorder called Rett syndrome (RTT). The syndrome was originally described in 1966 by the Austrian pediatrician Andreas Rett, but its genetic basis was revealed only recently (Amir et al. 1999). At least 80% of RTT cases are caused by spontaneous mutations in the MeCP2 gene (see Kriaucionis and Bird 2003), which is localized on Xq28 (Amir et al. 1999). RTT is the second most frequent form of female mental retardation after Down syndrome, and its incidence is approximately twofold higher than phenylketonuria (Jellinger 2003). RTT is diagnosed in 1:10,000–1:22,000 female births, with affected girls being heterozygous for the *MeCP2* mutation (Kriaucionis and Bird 2003); consequently, the phenotype is caused by the cells that do not express functional protein due to random inactivation of the X chromosome containing the wild-type copy of *MeCP2*. Most mutations found in RTT patients are located within the functional domains, i.e., within the MBD and the TRD of MECP2, but several mutations have also been found in the C-terminal region, where no concrete function has yet been assigned.

Recently, however, it was shown that the C-terminal domain of MeCP2 is crucial at compacting oligonucleosomes into dense higher order conformations in vitro (Georgel et al. 2003). Interestingly, this activity was found to be independent of CpG methylation of the oligonucleosomal arrays, which parallels the findings in human and chicken where MeCP2 binding was also found at non-methylated sites (see above) (Weitzel et al. 1997; Koch and Stratling 2004). Moreover, the C-terminal domain of MeCP2 was found to specifically bind to the group II WW domain found in the splicing factors formin-binding protein (FBP) and HYPC (Buschdorf and Stratling 2004). Although the functional role of this association has yet to be unraveled, various mutations within this C-terminal region were shown to correlate with a RTT phenotype. In mouse models for RTT, animals carrying mutations in the C-terminus generally exhibit a less-severe phenotype than those with a null mutation (Shahbazian et al. 2002a). Mice where MeCP2 was conditionally knocked out only in brain tissue yielded the same phenotype as that where the whole animal was affected, suggesting that the observable phenotype is largely due to a failure of proper brain development (Chen et al. 2001; Guy et al. 2001). Mutations in MeCP2, moreover, have been shown to correlate with phenotypes containing clinical features of X-linked mental retardation (Couvert et al. 2001), Angelman syndrome (Watson et al. 2001), and autism (Carney et al. 2003; Zappella et al. 2003). In conclusion, RTT is a good example illustrating that not only are the establishment and replication of methylation marks pivotal for a normal development—as is shown by the severe phenotypes caused by loss of Dnmt functions—but the correct translation of DNA methylation marks is a critical prerequisite for normal ontogeny.

5 Outlook

The establishment and stable maintenance of epigenetic marks on the genome at each cell division as well as the translation of this epigenetic information into genome expression and stability is crucial for development and differentiation. This role of epigenetic regulatory mechanisms in the realization of the genome has been clearly established by the finding of mutations affecting epigenetic regulators in human diseases (RTT and ICF syndrome) and the severity of phenotypes in animal models carrying mutations in the different components of these pathways. In addition, global and local changes in methylation patterns of the genome are found in most tumors and have, therefore, triggered intense research into their usage as new tumor diagnostic tools and therapeutic targets.

Another recently emerging and exciting area of research where manipulating epigenetic information is of fundamental importance is stem cell therapy and animal cloning. In a reversed way to differentiation, resetting or reprogramming of the epigenetic state of a differentiated donor cell appears to be one of the major difficulties in animal cloning by nuclear transfer (reviewed, e.g., in Shi et al. 2003). Besides having a fundamental impact for basic research, understanding the nature of epigenetic information and its plasticity in (adult/embryonic) stem cells is a key prerequisite for successful clinical applications of cell replacement therapies in regenerative medicine.

Acknowledgements Work in the author's laboratories is funded by the Volkswagenstiftung and the Deutsche Forschungsgemeinschaft.

References

Aapola U, Lyle R, Krohn K, Antonarakis SE, Peterson P (2001) Isolation and initial characterization of the mouse Dnmt3 l gene. Cytogenet Cell Genet 92:122–126

Aguirre-Arteta AM, Grunewald I, Cardoso MC, Leonhardt H (2000) Expression of an alternative Dnmt1 isoform during muscle differentiation. Cell Growth Differ 11:551–559

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23:185–188

Arber W, Linn S (1969) DNA modification and restriction. Annu Rev Biochem 38:467–500

Bachman KE, Rountree MR, Baylin SB (2001) Dnmt3a and Dnmt3b are transcriptional repressors that exhibit unique localization properties to heterochromatin. J Biol Chem 276:32282–32287

Balmer D, Goldstine J, Rao YM, LaSalle JM (2003) Elevated methyl-CpG-binding protein 2 expression is acquired during postnatal human brain development and is correlated with alternative polyadenylation. J Mol Med 81:61–68

Bell AC, Felsenfeld G (2000) Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature 405:482–485

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

Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M (1999) A mammalian protein with specific demethylase activity for mCpG DNA. Nature 397:579–583

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

Buschdorf JP, Stratling WH (2004) A WW domain binding region in methyl-CpG-binding protein MeCP2: impact on Rett syndrome. J Mol Med 82:135–143

Cardoso MC, Leonhardt H (1999) DNA methyltransferase is actively retained in the cytoplasm during early development. J Cell Biol 147:25–32

Carlson LL, Page AW, Bestor TH (1992) Properties and localization of DNA methyltransferase in preimplantation mouse embryos: implications for genomic imprinting. Genes Dev 6:2536–2541

Carney RM, Wolpert CM, Ravan SA, Shahbazian M, Ashley-Koch A, Cuccaro ML, Vance JM, Pericak-Vance MA (2003) Identification of MeCP2 mutations in a series of females with autistic disorder. Pediatr Neurol 28:205–211

Chen RZ, Akbarian S, Tudor M, Jaenisch R (2001) Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. Nat Genet 27:327–331

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 22:22

Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R, Greenberg ME (2003) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 302:885–889

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

Cohen DR, Matarazzo V, Palmer AM, Tu Y, Jeon OH, Pevsner J, Ronnett GV (2003) Expression of MeCP2 in olfactory receptor neurons is developmentally regulated and occurs before synaptogenesis. Mol Cell Neurosci 22:417–429

Couvert P, Bienvenu T, Aquaviva C, Poirier K, Moraine C, Gendrot C, Verloes A, Andres C, Le Fevre AC, Souville I, Steffann J, des Portes V, Ropers HH, Yntema HG, Fryns JP, Briault S, Chelly J, Cherif B (2001) MECP2 is highly mutated in X-linked mental retardation. Hum Mol Genet 10:941–946

Cremer T, Cremer C (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2:292–301

Cummings DJ, Tait A, Goddard JM (1974) Methylated bases in DNA from Paramecium aurelia. Biochim Biophys Acta 374:1–11

Detich N, Theberge J, Szyf M (2002) Promoter-specific activation and demethylation by MBD2/demethylase. J Biol Chem 277:35791–35794

Doerfler W (1983) DNA methylation and gene activity. Annu Rev Biochem 52:93–124

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

Easwaran HP, Leonhardt H, Cardoso MC (2005) Cell cycle markers for live cell analyses. Cell Cycle 4:453–455

Felsenfeld G, Groudine M (2003) Controlling the double helix. Nature 421:448–453

Fisher AG, Merkenschlager M (2002) Gene silencing, cell fate and nuclear organisation. Curr Opin Genet Dev 12:193–197

Fujita N, Takebayashi S, Okumura K, Kudo S, Chiba T, Saya H, Nakao M (1999) Methylation-mediated transcriptional silencing in euchromatin by methyl-CpG binding protein MBD1 isoforms. Mol Cell Biol 19:6415–6426

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

Gaudet F, Rideout WM 3rd, Meissner A, Dausman J, Leonhardt H, Jaenisch R (2004) Dnmt1 expression in pre- and postimplantation embryogenesis and the maintenance of IAP silencing. Mol Cell Biol 24:1640–1648

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

Gorovsky MA, Hattman S, Pleger GL (1973) (6N)Methyl adenine in the nuclear DNA of a eucaryote, Tetrahymena pyriformis. J Cell Biol 56:697–701

Guy J, Hendrich B, Holmes M, Martin JE, Bird A (2001) A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat Genet 27:322–326

Hansen RS, Wijmenga C, Luo P, Stanek AM, Canfield TK, Weemaes CM, Gartler SM (1999) The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. Proc Natl Acad Sci U S A 96:14412–14417

Harikrishnan KN, Chow MZ, Baker EK, Pal S, Bassal S, Brasacchio D, Wang L, Craig JM, Jones PL, Sif S, El-Osta A (2005) Brahma links the SWI/SNF chromatin-remodeling complex with MeCP2-dependent transcriptional silencing. Nat Genet 37:254–264

Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM (2000) CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature 405:486–489

Hattman S, Kenny C, Berger L, Pratt K (1978) Comparative study of DNA methylation in three unicellular eucaryotes. J Bacteriol 135:1156–1157

Hendrich B, Bird A (1998) Identification and characterization of a family of mammalian methyl-CpG binding proteins. Mol Cell Biol 18:6538–6547

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

Hendrich B, Abbott C, McQueen H, Chambers D, Cross S, Bird A (1999a) Genomic structure and chromosomal mapping of the murine and human Mbd1, Mbd2, Mbd3, and Mbd4 genes. Mamm Genome 10:906–912

Hendrich B, Hardeland U, Ng HH, Jiricny J, Bird A (1999b) The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 401:301–304

Hendrich B, Guy J, Ramsahoye B, Wilson VA, Bird A (2001) Closely related proteins MBD2 and MBD3 play distinctive but interacting roles in mouse development. Genes Dev 15:710–723

Holliday R, Pugh JE (1975) DNA modification mechanisms and gene activity during development. Science 187:226–232

Horike S, Cai S, Miyano M, Cheng JF, Kohwi-Shigematsu T (2005) Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. Nat Genet 37:31–40

Hotchkiss RD (1948) The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. J Biol Chem 175:315–332

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

Hutchins AS, Mullen AC, Lee HW, Sykes KJ, High FA, Hendrich BD, Bird AP, Reiner SL (2002) Gene silencing quantitatively controls the function of a developmental trans-activator. Mol Cell 10:81–91

Jellinger KA (2003) Rett syndrome—an update. J Neural Transm 110:681–701

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

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

Jost JP, Oakeley EJ, Zhu B, Benjamin D, Thiry S, Siegmann M, Jost YC (2001) 5-Methylcytosine DNA glycosylase participates in the genome-wide loss of DNA methylation occurring during mouse myoblast differentiation. Nucleic Acids Res 29:4452–4461

Jung BP, Jugloff DG, Zhang G, Logan R, Brown S, Eubanks JH (2003) The expression of methyl CpG binding factor MeCP2 correlates with cellular differentiation in the developing rat brain and in cultured cells. J Neurobiol 55:86–96

Kanduri C, Pant V, Loukinov D, Pugacheva E, Qi CF, Wolffe A, Ohlsson R, Lobanenkov VV (2000) Functional association of CTCF with the insulator upstream of the H19 gene is parent of origin-specific and methylation-sensitive. Curr Biol 10:853–856

Koch C, Stratling WH (2004) DNA binding of methyl-CpG-binding protein MeCP2 in human MCF7 cells. Biochemistry 43:5011–5021

Kriaucionis S, Bird A (2003) DNA methylation and Rett syndrome. Hum Mol Genet 12 Suppl 2:R221–R227

Kriaucionis S, Bird A (2004) The major form of MeCP2 has a novel N-terminus generated by alternative splicing. Nucleic Acids Res 32:1818–1823
Kumar S, Cheng X, Klimasauskas S, Mi S, Posfai J, Roberts RJ, Wilson GG (1994) The DNA (cytosine-5) methyltransferases. Nucleic Acids Res 22:1–10
Kunert N, Marhold J, Stanke J, Stach D, Lyko F (2003) A Dnmt2-like protein mediates DNA methylation in Drosophila. Development 130:5083–5090
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
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
Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell 69:905–914
Li E, Bestor TH, Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 69:915–926
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
Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun YE (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 302:890–893
Matarazzo V, Ronnett GV (2004) Temporal and regional differences in the olfactory proteome as a consequence of MeCP2 deficiency. Proc Natl Acad Sci U S A 101:7763–7768
Mayer W, Niveleau A, Walter J, Fundele R, Haaf T (2000) Demethylation of the zygotic paternal genome. Nature 403:501–502
Meehan RR, Lewis JD, McKay S, Kleiner EL, Bird AP (1989) Identification of a mammalian protein that binds specifically to DNA containing methylated CpGs. Cell 58:499–507
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
Millar CB, Guy J, Sansom OJ, Selfridge J, MacDougall E, Hendrich B, Keightley PD, Bishop SM, Clarke AR, Bird A (2002) Enhanced CpG mutability and tumorigenesis in MBD4-deficient mice. Science 297:403–405
Mnatzakanian GN, Lohi H, Munteanu I, Alfred SE, Yamada T, MacLeod PJ, Jones JR, Scherer SW, Schanen NC, Friez MJ, Vincent JB, Minassian BA (2004) A previously unidentified MECP2 open reading frame defines a new protein isoform relevant to Rett syndrome. Nat Genet 36:339–341
Mullaney BC, Johnston MV, Blue ME (2004) Developmental expression of methyl-CpG binding protein 2 is dynamically regulated in the rodent brain. Neuroscience 123:939–949

Nan X, Campoy FJ, Bird A (1997) MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell 88:471–481

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

Ng HH, Zhang Y, Hendrich B, Johnson CA, Turner BM, Erdjument-Bromage H, Tempst P, Reinberg D, Bird A (1999) MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. Nat Genet 23:58–61

Ng HH, Jeppesen P, Bird A (2000) Active repression of methylated genes by the chromosomal protein MBD1. Mol Cell Biol 20:1394–1406

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

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

Pradhan S, Talbot D, Sha M, Benner J, Hornstra L, Li E, Jaenisch R, Roberts RJ (1997) Baculovirus-mediated expression and characterization of the full-length murine DNA methyltransferase. Nucleic Acids Res 25:4666–4673

Prokhortchouk A, Hendrich B, Jorgensen H, Ruzov A, Wilm M, Georgiev G, Bird A, Prokhortchouk E (2001) The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. Genes Dev 15:1613–1618

Riggs AD (1975) X inactivation, differentiation, and DNA methylation. Cytogenet Cell Genet 14:9–25

Robertson KD (2002) DNA methylation and chromatin—unraveling the tangled web. Oncogene 21:5361–5379

Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, Jones PA (1999) The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. Nucleic Acids Res 27:2291–2298

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

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

Ruzov A, Dunican DS, Prokhortchouk A, Pennings S, Stancheva I, Prokhortchouk E, Meehan RR (2004) Kaiso is a genome-wide repressor of transcription that is essential for amphibian development. Development 131:6185–6194

Saito M, Ishikawa F (2002) The mCpG-binding domain of human MBD3 does not bind to mCpG but interacts with NuRD/Mi2 components HDAC1 and MTA2. J Biol Chem 277:35434–35439

Selker EU (1997) Epigenetic phenomena in filamentous fungi: useful paradigms or repeat-induced confusion? Trends Genet 13:296–301

Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J, Armstrong D, Paylor R, Zoghbi H (2002a) Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. Neuron 35:243–254

Shahbazian MD, Antalffy B, Armstrong DL, Zoghbi HY (2002b) Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. Hum Mol Genet 11:115–124

Shi W, Zakhartchenko V, Wolf E (2003) Epigenetic reprogramming in mammalian nuclear transfer. Differentiation 71:91–113

Spector DL (2003) The dynamics of chromosome organization and gene regulation. Annu Rev Biochem 72:573–608

Srinivasan PR, Borek E (1964) Enzymatic alteration of nucleic acid structure. Science 145:548–553

Stancheva I, Collins AL, Van den Veyver IB, Zoghbi H, Meehan RR (2003) A mutant form of MeCP2 protein associated with human Rett syndrome cannot be displaced from methylated DNA by notch in Xenopus embryos. Mol Cell 12:425–435

Szabo P, Tang SH, Rentsendorj A, Pfeifer GP, Mann JR (2000) Maternal-specific footprints at putative CTCF sites in the H19 imprinting control region give evidence for insulator function. Curr Biol 10:607–610

Tariq M, Paszkowski J (2004) DNA and histone methylation in plants. Trends Genet 20:244–251

Tucker KL, Talbot D, Lee MA, Leonhardt H, Jaenisch R (1996) Complementation of methylation deficiency in embryonic stem cells by a DNA methyltransferase minigene. Proc Natl Acad Sci USA 93:12920–12925

Tudor M, Akbarian S, Chen RZ, Jaenisch R (2002) Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. Proc Natl Acad Sci U S A 99:15536–15541

Tweedie S, Charlton J, Clark V, Bird A (1997) Methylation of genomes and genes at the invertebrate-vertebrate boundary. Mol Cell Biol 17:1469–1475

Vairapandi M (2004) Characterization of DNA demethylation in normal and cancerous cell lines and the regulatory role of cell cycle proteins in human DNA demethylase activity. J Cell Biochem 91:572–583

von Kries JP, Buhrmester H, Stratling WH (1991) A matrix/scaffold attachment region binding protein: identification, purification, and mode of binding. Cell 64:123–135

Wade PA (2001) Methyl CpG binding proteins: coupling chromatin architecture to gene regulation. Oncogene 20:3166–3173

Wade PA, Gegonne A, Jones PL, Ballestar E, Aubry F, Wolffe AP (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat Genet 23:62–66

Watson P, Black G, Ramsden S, Barrow M, Super M, Kerr B, Clayton-Smith J (2001) Angelman syndrome phenotype associated with mutations in MECP2, a gene encoding a methyl CpG binding protein. J Med Genet 38:224–228

Weitzel JM, Buhrmester H, Stratling WH (1997) Chicken MAR-binding protein ARBP is homologous to rat methyl-CpG-binding protein MeCP2. Mol Cell Biol 17:5656–5666

Wong E, Yang K, Kuraguchi M, Werling U, Avdievich E, Fan K, Fazzari M, Jin B, Brown AM, Lipkin M, Edelmann W (2002) Mbd4 inactivation increases Crightarrow T transition mutations and promotes gastrointestinal tumor formation. Proc Natl Acad Sci U S A 99:14937–14942

Wyatt GR (1951) Recognition and estimation of 5-methylcytosine in nucleic acids. J Biochem (Tokyo) 48:581–584

Xie S, Wang Z, Okano M, Nogami M, Li Y, He WW, Okumura K, Li E (1999) Cloning, expression and chromosome locations of the human DNMT3 gene family. Gene 236:87–95

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

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

Yoder JA, Yen RWC, Vertino PM, Bestor TH, Baylin SB (1996) New 5' regions of the murine and human genes for DNA (cytosine-5)-methyltransferase. J Biol Chem 271:31092–31097

Zappella M, Meloni I, Longo I, Canitano R, Hayek G, Rosaia L, Mari F, Renieri A (2003) Study of MECP2 gene in Rett syndrome variants and autistic girls. Am J Med Genet B Neuropsychiatr Genet 119:102–107

Zhao X, Ueba T, Christie BR, Barkho B, McConnell MJ, Nakashima K, Lein ES, Eadie BD, Willhoite AR, Muotri AR, Summers RG, Chun J, Lee KF, Gage FH (2003) Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. Proc Natl Acad Sci U S A 100:6777–6782