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REVIEW

DNA methylation and methyl-CpG binding proteins: developmental requirements and function

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Abstract DNA methylation is a major epigenetic modification in the genomes of higher eukaryotes. In vertebrates, DNA methylation occurs predominantly on the CpG dinucleotide, and approximately 60% to 90% of these dinucleotides are modified. Distinct DNA methylation patterns, which can vary between different tissues and developmental stages, exist on specific loci. Sites of DNA methylation are occupied by various proteins, including methyl-CpG binding domain (MBD) proteins which recruit the enzymatic machinery to establish silent chromatin. Mutations in the MBD family member MeCP2 are the cause of Rett syndrome, a severe neurodevelopmental disorder, whereas other MBDs are known to bind sites of hypermethylation in human cancer cell lines. Here, we review the advances in our understanding of the function of DNA methylation, DNA methyltransferases, and methyl-CpG binding proteins in vertebrate embryonic development. MBDs function in transcriptional repression and long-range interactions in chromatin and also appear to play a role in genomic stability, neural signaling, and transcriptional activation. DNA methylation makes an essential and versatile epigenetic contribution to genome integrity and function.

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Introduction

DNA methylation is a covalent modification of DNA catalyzed by DNA methyltransferase enzymes (DNMTs). In vertebrate genomes, the addition of a methyl group occurs exclusively on the cytosine within CG dinucleotides (referred to as CpG), with 60–90% of all the CpGs methylated in mammals (Bird 1986). The exceptions are CpG islands, CpG-enriched sequences that frequently coincide with gene promoter regions and generally are unmethylated. DNA methylation in higher eukaryotes is usually associated with a repressed chromatin environment, while in the prokaryote kingdom both cytosine and adenine methylation have been described as a part of the host restriction system (Wilson and Murray 1991). Proper DNA methylation is a prerequisite for normal development and is involved in various processes such as gene repression, imprinting, X-chromosome inactivation, suppression of repetitive genomic elements, and carcinogenesis (Bird 2002). Sites of DNA methylation recruit methyl-CpG binding domain proteins (MBDs) and several structurally unrelated methyl-CpG binding zinc-finger proteins of the Kaiso family (Kaiso/ZBTB33, ZBTB4 and ZBTB38, cf. Fig. 1). These proteins generally are thought to associate with histone deacetylase activity and establish silent chromatin (Table 1). Here, we review the recent developments in the field of DNA methylation-dependent silencing with special emphasis on the role of MBDs in vertebrate development. DNA methylation, MBDs, and their roles in disease have been more extensively reviewed elsewhere (Clouaire and Stancheva 2008; Hendrich and Tweedie 2003; Klose and Bird 2006; Lopez-Serra and Esteller 2008).

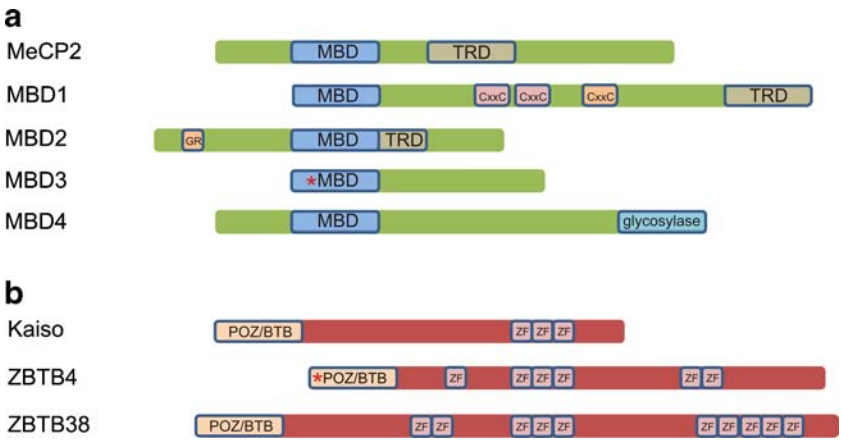

Fig. 1 Two families of proteins that bind methylated DNA. **a** Methyl-CpG binding proteins (*MBDs*): MBD proteins display homology within their MBD domains, while the transcription repression domains (*TRD*) described for MeCP2, MBD1, and MBD2 are non-homologous. In addition to its MBD domain, MBD1 is able to bind unmethylated DNA via its third CxxC zinc-finger motif. MBD2 features a characteristic stretch of glycine and arginine residues (*GR*) and has juxtaposed MBD and TRD domains. MBD3 is, due to a mutation in the MBD domain, not able to bind methylated CpGs in mammals. MBD4, a thymine glycosylase, contains a C-terminal glycosylase domain used for excision-based DNA repair. **b** Kaiso protein family: three members of the Kaiso protein family have been described so far. Kaiso, ZBTB4, and ZBTB38 share a triple zinc-finger domain and a BTB/POZ domain which in case of ZBTB4 contains a 60 amino acid insertion. Furthermore, ZBTB4 and ZBTB38 contain respectively three and seven additional zinc-fingers

DNA methylation: distribution and dynamics

DNA methylation in different species

Vertebrate genomes are highly methylated, but many invertebrates have a low genomic DNA methylation content and a few species like *Caenorhabditis elegans* and *Drosophila melanogaster* appear (virtually) methylation-free (Tweedie et al. 1997). Likewise, both *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* are devoid of DNA methylation (Antequera et al. 1984; Proffitt et al. 1984), but the filamentous fungus *Neurospora crassa* utilizes a silencing pathway in which the establishment of DNA methylation is dependent on the H3K9 histone-methyltransferase *dim-5* (Tamaru and Selker 2001). A study mapping DNA methylation in the *Neurospora* genome revealed that most of the methylated sequences correspond to transposon relics (Selker et al. 2003), in line with a role for DNA methylation in preventing the reactivation of parasitic genomic sequences in eukaryotes (Bestor and Tycko 1996; Yoder et al. 1997). Species such as *D. melanogaster* which have very little DNA methylation display a relatively high mutation rate due to the vulnerability of their genome to genomic transposition (Yoder et al. 1997). Although the DNA methylation mark does not seem to be particularly abundant in *Drosophila*, two potential DNA methyltransferase genes have been discovered (Hung et al. 1999). In contrast to the vertebrate DNMT2, which has mostly been associated with RNA methyltransferase activity (Goll et al. 2006; Rai et al. 2007), deletion of *Dnmt2* from the fly genome resulted in abolishment of DNA methylation whereas its overexpression induced hypermethylation on CpT and CpA dinucleotides (Kunert et al. 2003). A single functional homolog of the mammalian MBD2 and MBD3 proteins has also been discovered in *Drosophila* (Roder et al. 2000). GST pull-downs as well as yeast two-hybrid assays showed that fly MBD2/3 protein interacts with the Mi-2/NuRD complex via the p55 and Mi-2 subunits (Marhold et al. 2004a). The generation of the MBD2/3 mutant allele resulted in viable and fertile flies which however showed some displacement of Mi-2 from genomic loci (Marhold et al. 2004b). Band-shifts using MBD2/3 and its mammalian MBD2 homolog demonstrated that fly MBD2/3 interacts with CpT/A methylated, but not CpG-methylated oligonucleotides, while the mammalian MBD2 interacted only with the CpG methylated probes. Also, immunohistochemistry experiments suggest that embryos treated with the DNA methylation inhibitor 5-azacytidine display a loss of MBD2/3 foci which normally overlap with the DNA staining (Marhold et al. 2004b). Collectively, these data indicate that *Drosophila* MBD2/3 might be functionally more similar to MBD2 than to MBD3. However, further experiments will be needed in order to determine whether MBD2/3 targets the Mi-2/NuRD complex to the sites of CpT/A methylation or if such a complex is mainly targeted by protein–protein interactions. It is possible that DNA methylation only plays an auxiliary role, for example, during development; the 5-methylcytosine content of the *Drosophila* genome as assessed by liquid chromatography showed the highest signal in the early embryo (Lyko et al. 2000).While the *Drosophila* genome

Table 1 Biochemical interactions of methyl CpG binding proteins

Protein	Interacting partner	Effects of the interaction	Reference
MeCP2	Sin3A, HDACs	Transcriptional repression	Jones et al. (1998), Nan et al. (1998)
	c-ski, N-CoR	Transcriptional repression	Kokura et al. (2001)
	HMGB1	Unknown	Dintilhac and Bernues (2002)
	Sin3B, HDAC2	Transcriptional repression	Rietveld et al. (2002)
	Dnmt1	Targeting of maintenance DNA methylation?	Kimura and Shiota (2003)
	H3K9 methyltransferase	Transcriptional repression	Fuks et al. (2003b)
	CoREST complex	Repression of neural genes	Ballas et al. (2005), Lunyak et al. (2002)
	Brm (SWI/SNF complex)	Transcriptional repression	Harikrishnan et al. (2005), Hu et al. (2006)
	YB-1	Alternative splicing	Young et al. (2005)
	ATRX	Epigenetic regulation required for neural development	Nan et al. (2007)
	HP1	Transcriptional repression during myogenic differentiation	Agarwal et al. (2007)
	CREB1	Transcriptional activation	Chahrour et al. (2008)
MBD1	MPG	DNA repair	Watanabe et al. (2003)
	Suv39h1-HP1	Transcriptional repression	Fujita et al. (2003)
	MCAF1, MCAF2, SETDB1, CAF-1 p150	Transcriptional repression, inheritance of epigenetic states	Ichimura et al. (2005), Reese et al. (2003), Sarraf and Stancheva (2004)
	PML-RARα, HDAC3	PML-RARα-mediated silencing	Villa et al. (2006)
MBD2	Mi-2, MTA1-3, P66α/β, HDAC1/2, RbAp46/48, DOC-1, PRMT5, MEP50 (NuRD complex)	Transcriptional repression	Brackertz et al. (2002), Le Guezennec et al. (2006), Zhang et al. (1999)
	Sin3A	Transcriptional repression	Boeke et al. (2000)
	Tax	Transcriptional activation	Ego et al. (2005)
	TACC3, HATs, pCAF	Transcriptional activation	Angrisano et al. (2006)
	GCNF	Oct-4 silencing	Gu et al. (2006)
	Dnmt1	Targeting of maintenance DNA methylation?	Tatematsu et al. (2000)
	RFP	Enhancement of transcriptional repression	Fukushige et al. (2006)
MBD3	Mi-2, MTA1-3, P66α/β, HDAC1/2, RbAp46/48, DOC-1 (NuRD complex)	Transcriptional repression	Le Guezennec et al. (2006), Wade et al. (1999), Zhang et al. (1999)
	Dnmt1	Targeting of maintenance DNA methylation?	Tatematsu et al. (2000)
	CDK2AP1, GCNF	Oct-4 silencing	Deshpande et al. (2009), Gu et al. (2006)
MBD4	Sin3A, HDAC1	Transcriptional repression	Kondo et al. (2005)
	FADD	Genome surveillance/apoptosis?	Screaton et al. (2003)
	MLH1	DNA repair	Bellacosa et al. (1999)
	RFP	Enhancement of transcriptional repression	Fukushige et al. (2006)
Kaiso	Tcf3	Suppression of Wnt signaling	Ruzov et al. (2009)
	p120	Wnt signaling?	Daniel and Reynolds (1999), Prokhortchouk et al. (2001)
	N-CoR	Transcriptional repression	Yoon et al. (2003)

appears to be largely depleted of DNA methylation, other insect species such as the cabbage moth *Mamestra brassicae*, the peach potato aphid *Myzus persicae*, and the mealy bug *Planococcus citri* seem to utilize DNA methylation (Field et al. 2004). Also, significant amounts of DNA methylation along with a MBD2/3 homolog have been identified in the silkworm moth *Bombyx mori* (Patel and Gopinathan 1987; Uno et al. 2005). A functional DNA methylation system containing both Dnmt1 and Dnmt3 as well as a functional ortholog of the mammalian MBD family has been described in the honeybee, *Apis melifera* (Wang et al. 2006). In the honeybee, DNA methylation plays an

important role in the organization of social structures as well as labor division (Kucharski et al. 2008). In bee communities, young worker bees feed a privileged subset of larvae with a substance called royal jelly (Schmitzova et al. 1998). Larvae nurtured with royal jelly develop into queens, while other bees of the same clonal origin become worker bees (Barchuk et al. 2007; Colhoun and Smith 1960). The larvae treated with small interfering RNA targeting a de novo DNA methyltransferase, Dnmt3, developed into queens with fully functional ovaries, while a control RNA did not induce that effect (Kucharski et al. 2008). This finding highlights the function of DNA methylation in phenotypic plasticity and also shows an elegant way of how epigenomes respond to environmental signals in order to determine different developmental fates. Vertebrates such as *Xenopus laevis* and zebrafish, *Danio rerio*, are known to have a high content of genomic DNA methylation as well as functional DNMTs and MBDs (McGowan and Martin 1997; Rai et al. 2006; Stancheva and Meehan 2000; Veenstra and Wolffe 2001). DNA methylation is also employed in mammal-specific ways. A phenomenon observed in both eutherians and marsupials is parental imprinting, which is allele-specific gene expression in which the expression status of an allele is determined by its parental origin (Reik et al. 2001; Wood and Oakey 2006). The differential expression status of maternal and paternal alleles is controlled by DNA methylation. Defects in imprinting are related with severe diseases such as the Beckwith–Wiedemann, Prader–Willi, and Angelman syndromes and various types of cancer (Bittel and Butler 2005; Lalande and Calciano 2007; Rainier et al. 1993; Weksberg et al. 2003). The imprints are established already during gametogenesis (Bourc'his et al. 2001; Delaval and Feil 2004). Developmental dynamics of DNA methylation and its function in vertebrates will be discussed in more detail below.

DNA methyltransferases in embryonic development

The DNA methylation mark in vertebrates is set by three DNMT family members: DNMT1, DNMT3a, and DNMT3b. DNMT3a and DNMT3b fall in the group of de novo methyltransferases, enzymes that are able to methylate previously unmethylated CpG sequences, while DNMT1 functions as a maintenance methylase, copying the pre-existing methylation marks onto the new strand during replication (Jeltsch 2006). Although generally thought of as a maintenance methylase, DNMT1 has also been shown to function as a de novo DNA methyltransferase (Fatemi et al. 2002; Gowher et al. 2005; Liang et al. 2002; Pradhan et al. 1999). In addition, two non-canonical family members, DNMT2 and DNMT3L, have been discovered (Aapola et al. 2000; Okano et al. 1998). Various functional studies demonstrated the importance of DNMTs for early vertebrate development (Li et al. 1992; Okano et al. 1999; Stancheva et al. 2001).

The DNMT1 gene was targeted in embryonic stem (ES) cells and in mouse embryos (Li et al. 1992). Both affected ES cells and embryos showed significantly reduced levels of DNA methylation. The loss of DNMT1 proved to be lethal with the majority of embryos not passing mid-gestation, although the ES cells remained viable and proliferative (Li et al. 1992). Dnmt1o, a variant transcribed from an oocyte-specific promoter, expressed in oocytes and preimplantation embryos, is required for zygotic maintenance of imprinting (Howell et al. 2001). In *X. laevis* embryos, the genome of DNMT1-depleted embryos was hypomethylated and the embryos displayed a premature expression pattern of several mesodermal markers. (Stancheva and Meehan 2000). This also led to p53-induced apoptosis and embryonic lethality (Stancheva et al. 2001). Similar observations have been made on cultured fibroblasts derived from conditional DNMT1 mouse knockouts that undergo p53-dependent programmed cell death (Jackson-Grusby et al. 2001). The DNMT1-depleted fibroblasts showed a reactivation of placental and germ line markers pointing out the role of DNMT1 for tissue-specific gene expression and embryonic development. The DNMT1 knockdown in zebrafish (Rai et al. 2006) appears to recapitulate the effects observed in mice and *Xenopus* with ~40% of the embryos dying upon DNMT1 depletion. An interesting finding was that the response to DNMT1 deficiency was largely organ-specific. One of the most affected organs was the gut, where the reduced intestinal differentiation was accompanied by the loss of expression of *fabp2*, a marker of terminally differentiated epithelial cells. Although markers of eye development such as *otx-2* and *otx-5* appeared to be expressed at similar levels in both control embryos and DNMT1 morphants, histological evidence suggested a severe disorganization of retinal structures. This study emphasizes the importance of DNMT1 for tissue-specific gene expression and development. DNMT1 has been reported to interact with methyl-CpG binding proteins as well as with HDACs and histone methyltransferases to repress transcription (Fuks et al. 2003a; Kimura and Shiota 2003; Tatematsu et al. 2000). In addition, DNMT1 has been found to interact with the Rb tumor suppressor protein to repress transcription from promoters containing E2F binding sites (Robertson et al. 2000), linking DNMT1 to a growth regulatory pathway that frequently is disrupted in cancer.

DNMT2 is the best conserved methyltransferase although its exact function remains a topic of debate. DNMT2 appears to be dispensable for de novo DNA methylation in mouse ES cells (Okano et al. 1998), while in vitro experiments detected only a weak methyltransferase activity (Hermann et al. 2003). Interestingly, a recent study

found that DNMT2 can function as a tRNA methyltransferase that specifically methylates cytosine 38 in the anticodon loop (Goll et al. 2006). Although the exact function of this cytoplasmic methylation event remains unclear, another study described the requirement for DNMT2 cytoplasmic activity in early zebrafish development (Rai et al. 2007). The DNMT2 knockdown was not lethal; however DNMT2 morphant embryos displayed a range of developmental defects including brain and retina abnormalities. DNMT2 isolated from zebrafish was able to methylate an RNA species of ~80 bp, which by size might correspond to transfer RNA (tRNA). It is not known how many RNA species can be methylated by cytoplasmic DNMT2.

DNMT3a and DNMT3b targeting in mice revealed that both de novo DNA methyltransferases are essential for early mouse development (Okano et al. 1999). Although the expression patterns of DNMT3a and DNMT3b are largely overlapping, the functions that they carry out do not seem to be completely redundant since both knockouts turned out to be lethal. DNMT3a-depleted mice appeared normal after birth but died at 4 weeks of age. On the other hand, no DNMT3b knockouts were recovered at birth. The double knockout induced a more severe phenotype since the affected embryos showed developmental defects at E8.5 and died shortly after gastrulation. Furthermore, both DNMT3a and DNMT3b proved to be essential for the lineage-specific DNA methylation of Rhox6 and Rhox9 cluster genes, with DNMT3b contributing slightly more to the amount of methylation established (Oda et al. 2006). Taken together, these data indicate that some but not all of the functions are shared by both enzymes. This finds further support in the fact that mutations in DNMT3b, but not DNMT3a, cause a recessive autosomal disorder called immunodeficiency, centromeric instability, and facial abnormalities (ICF) syndrome. ICF syndrome is associated with hypomethylation of satellites II and III which results in expansion of juxtacentromeric heterochromatin and formation of complex multiradiate chromosomes (Xu et al. 1999). Besides the DNMT3a and DNMT3b methyltransferases, another family member has been described in the last couple of years. DNMT3L is a catalytically inactive DNMT which is known to associate with both DNMT3a and DNMT3b to establish regions of maternal imprinting (Hata et al. 2002). Furthermore, DNMT3L is able to recruit histone deacetylases through its PHD zinc-finger-like motif and possibly directs repression onto newly established imprints (Aapola et al. 2002; Deplus et al. 2002).

Dynamic changes in DNA methylation during vertebrate development

Although stable and inheritable in somatic cells, global DNA methylation patterns are dynamic during the mammalian life cycle. Global remodeling of DNA methylation occurs twice in mammals, during gametogenesis and preimplantation development (Morgan et al. 2005). The first erasure of DNA methylation marks takes place during gametogenesis, when also the imprinted marks are reset. This involves a wave of remethylation which is needed to establish the parental imprints. The second demethylation event takes place during preimplantation development and does not affect imprinted regions (Mann and Bartolomei 2002). When compared to the oocyte genome, the sperm genome is highly methylated, which correlates well with its inactive chromatin state and compact structure (Morgan et al. 2005). Immunohistochemistry and bisulfate conversion experiments in mice showed that the male pronucleus gets rapidly demethylated shortly after fertilization (Mayer et al. 2000; Oswald et al. 2000), while the maternal genome displays a slow but progressive drop in DNA methylation levels consistent with passive demethylation. Later during implantation, the global DNA methylation levels of both the paternal and the maternal contributions to the genome increase (Meehan 2003). Such developmental changes in the DNA methylation content of the embryo have been described in other placental mammals as well (Dean et al. 2001). By contrast, the *Xenopus* paternal genome does not get actively demethylated after fertilization despite the occurring changes in the chromatin structure (Stancheva et al. 2002). Moreover, the global DNA methylation content as well as DNA methylation of specific loci remains high and unchanged during embryogenesis from early blastula stages onwards (Veenstra and Wolffe 2001). However, some DNA methylation remodeling has been reported for a number of developmentally regulated promoters (Stancheva et al. 2002). Likewise, southern blotting of repetitive DNA and bisulfate sequencing of single copy genes demonstrated the absence of global demethylation in zebrafish embryonic development (Macleod et al. 1999). Immunohistochemistry experiments with an anti-5-methylcytosine antibody, however, showed a reduction in signal to almost undetectable levels 1.5–2 h post fertilization (hpf) and a reappearance at 2.5 hpf (MacKay et al. 2007). Possible explanations for this discrepancy include (subtle) differences in the stages and sequences analyzed and methodological differences in detection of DNA methylation. A demethylase activity involving an AID deaminase, MBD4 glycosylase, and Gadd45a has recently been reported in late gastrula to segmentation stage (8–13 hpf) zebrafish embryos (Rai et al. 2008). Gadd45a has been associated with demethylation events (Barreto et al. 2007; Schmitz et al. 2009). However, Gadd45a knockout in mice did not result in global or site-specific hypermethylation (Engel et al. 2009) and the role of Gadd45a in demethylation is disputed (Jin et al. 2008). An equally controversial DNA demethylase function has been proposed for the MBD2 and MBD3 proteins

(Bhattacharya et al. 1999; Boeke et al. 2000; Brown et al. 2008; Detich et al. 2002; Hendrich et al. 2001; Ng et al. 1999; Wade et al. 1999; Wolffe et al. 1999). To what extent the observations concerning active demethylation can be reconciled, and how DNA methylation and demethylation are targeted or regulated, and what the physiological role of DNA demethylation is in vertebrate development, are issues that await further clarification.

DNA methylation and histone modifications provide a link between chromatin and tissue-specific transcriptional programs (Kiefer 2007; Lunyak et al. 2004; Palacios and Puri 2006; Rupp et al. 2002). Until recently, most of the DNA methylation studies have been performed on individual genomic loci. However, new high-throughput technologies have allowed us to investigate thousands of CpG methylation sites across the genome and collect tissue-specific as well as age- and sex-dependent DNA methylation signatures. A bisulfate conversion approach combined with BeadArray technology identified DNA methylation epigenotypes specific for distinct cell populations (Bibikova et al. 2006a; Bibikova et al. 2006b). Out of 1,536 CpG sites selected from 5′ regulatory regions of 371 genes, 49 sites from 40 genes were identified as potential DNA methylation markers. The differential DNA methylation patterns observed on those sites were sufficient to discriminate between human ES cells, differentiated cells, somatic cells, and cancer cells. Another study using bisulfate DNA sequencing of chromosomes 6, 20 and 22 on samples derived from 12 different healthy tissues, including fetal liver and fetal skeletal muscle, identified a number of genes carrying DNA methylation patterns specific to fetal tissues (Eckhardt et al. 2006), while a relatively new technique which uses the combination of methylated DNA immunoprecipitation (MeDIP) and microarray hybridization proved to be quite efficient in generating global DNA methylation data and assessing the differences between normal and transformed cells (Weber et al. 2005). When compared to primary fibroblasts and normal colon mucosa, the SW48 colon carcinoma cell line was found to be hypermethylated at ~200 unique genes. The same study also revealed hypomethylation of the inactive X chromosome when compared to its active counterpart while hypermethylation was detectable only on a subset of gene-rich regions (Weber et al. 2005). “Next generation” sequencing tools combined with methylation-specific enzyme digestion also found their application in global DNA methylation profiling (Brunner et al. 2009). The study revealed minimal, although significant, changes in DNA methylation content during human liver differentiation. While the in vivo fetal liver development was characterized by a slight decrease in DNA methylation, the in vitro differentiation of human ES cells was marked by both de novo methylation and demethylation events (Brunner et al. 2009). In conclusion, distinct DNA methylation patterns exist on specific genomic loci, depending on lineage and differentiation potential.

Methyl-CpG binding proteins and embryonic development

Generally, two different mechanisms by which DNA methylation represses gene expression have been identified. A first, direct mechanism is based on the alteration of transcription factor binding sites by DNA methylation. A methylated target sequence can interfere with the binding of transcription factors such as E2F or CREB, thereby preventing transcriptional activation (Campanero et al. 2000; Iguchi-Ariga and Schaffner 1989). A more elaborate mechanism features recruitment of methyl-CpG binding proteins which associate with various chromatin modifiers to establish a repressive chromatin environment (Fuks et al. 2003b; Jones et al. 1998; Nan et al. 1998; Wade et al. 1999; Zhang et al. 1999). These proteins read and interpret the epigenetic signals and provide a connection between DNA methylation and chromatin modification. In this section, we will review proteins that feature a MBD and the structurally unrelated zinc-finger proteins sharing a BTB/POZ domain that are known to bind methylated DNA (Fig. 1). The MBD family proteins have been comprehensively studied through the years and their characterization unraveled specific functions carried out by each family member (see also Tables 1 and 2). Mutations in the MBD family founder, methyl-CpG binding protein 2 (MeCP2), are the cause of the Rett syndrome (RTT), an X-linked neurodevelopmental disorder (Amir et al. 1999). Other MBD family proteins have been shown to bind aberrantly hypermethylated promoters in various human cancer cell lines (Ballestar et al. 2003). The MBD was initially identified as the minimal part of the MeCP2 protein required to bind methylated DNA (Nan et al. 1993), and homology searches with the MeCP2 MBD amino acid sequence led to the discovery of four additional proteins, MBD1, MBD2, MBD3, and MBD4 (Hendrich and Bird 1998). A non-conserved transcription repression domain has been identified in MeCP2, MBD1, and MBD2 (Fig. 1). Apart from its MBD domain, MBD1 protein can bind DNA via its CxxC3 zinc-finger domain (Jorgensen et al. 2004), which shows noticeable sequence resemblance with the CxxC domains of DNMT1 (Pradhan et al. 2008). All MBD proteins preferentially bind methylated rather than unmethylated DNA, except mammalian MBD3 (Saito and Ishikawa 2002) and the long form of the amphibian MBD3 (MBD3 LF), which due to an insertion in the MBD, is not able to specifically recognize DNA methylation (Wade et al. 1999). Generally, the affinity of MBD proteins for methylated

Table 2 Phenotypes caused by loss of function of methyl-CpG binding proteins

Protein	Model system	Experimental approach	Phenotype	Reference
MeCP2	*Mus musculus*	Knockout	Neural, RTT-like phenotype	Guy et al. (2001)
	Xenopus laevis	Antisense knockdown	Improper neural patterning, embryonically lethal	Stancheva et al. (2003)
MBD1	*Mus musculus*	Knockout	Minor neural defects, increased genomic instability	Zhao et al. (2003)
MBD2	*Mus musculus*	Knockout	Mild maternal phenotype, abnormal differentiation, reduced tumorigenesis	Hendrich et al. (2001), Sansom et al. (2003)
MBD3	*Mus musculus*	Knockout	Failure in differentiation of pluripotent cells embryonically lethal	Hendrich et al. (2001), Kaji et al. (2007)
	Xenopus laevis	Antisense knockdown	Defective eye formation, embryonically lethal	Iwano et al. (2004)
MBD4	*Mus musculus*	Knockout	No apparent phenotype, increased mutation rate	Millar et al. (2002), Wong et al. (2002)
Kaiso	*Mus musculus*	Knockout	No apparent phenotype, reduced tumorigenesis	Prokhortchouk et al. (2006)
	Xenopus laevis	Antisense knockdown	Premature activation of zygotic transcription	Ruzov et al. (2004)

DNA is three- to tenfold higher than for unmethylated DNA and may as well depend on the sequence context (Fraga et al. 2003). Experiments involving in vitro binding site selection revealed a requirement of human MeCP2 for an A/T-rich sequence following the CpG methylation site (Klose et al. 2005). Apart from the well-studied MBD family, other proteins have been identified that preferentially bind methylated DNA (Arita et al. 2008; Avvakumov et al. 2008; Daniel et al. 2002; Filion et al. 2006; Prokhortchouk et al. 2001; Sharif et al. 2007; Woo et al. 2007).

MeCP2

Several molecular roles have been described for MeCP2: transcriptional repression, activation of transcription, nuclear organization, and splicing. These roles will be elaborated further in the following sections. Alongside with different functions, myriad interactions have been reported (Table 1). Most of these interactions have been isolated under low stringency conditions as MeCP2 does not form a single stable complex (Klose and Bird 2004), but may interact with other proteins in a context-dependent fashion. It is probably the flexible secondary and tertiary structure of the MeCP2 protein which allows it to carry out so many diverse functions, as MeCP2 appears to be a highly disordered protein (Adams et al. 2007).

Transcriptional repression by MeCP2

In addition to binding methylated DNA, MeCP2 associates with various co-repressor complexes such as Sin3a, NCoR, and c-Ski at the sites of its occupance (Jones et al. 1998; Kokura et al. 2001). When targeted to promoter DNA, MeCP2 causes strong transcriptional repression, suggesting that MeCP2 might serve as a global transcriptional silencer (Jones et al. 1998; Nan et al. 1998). Transcriptional profiling of *MeCP2*-null mice brains, however, displayed only subtle changes in gene expression (Tudor et al. 2002). Such a finding was confirmed in a later study where RNA isolated from the cerebellum of MeCP2 mutant mice has been subjected to microarray hybridization (Jordan et al. 2007). Interestingly, special AT-rich sequence binding protein 1 (*SATB1*) was identified as one of the few genes upregulated in two *MeCP2*-null mouse models. SATB1 is known to specifically bind to nuclear matrix attachment regions (MARs) and mediate formation of chromatin loops, a feature that has been attributed to the MeCP2 protein as well (Horike et al. 2005; Weitzel et al. 1997). The most considerable change in expression was attributed to the *Irak1* gene, which showed a twofold increase in expression. Likewise, a global expression study using cultured fibroblast cell lines from two RTT patients revealed only 49 upregulated and 21 downregulated potential MeCP2 targets, some of which were known to be expressed in brain tissues (Traynor et al. 2002). However, striking differences were observed in different clones obtained from the same RTT patients, which may be indicative of epigenetic instability. Collectively, these data may indicate that MeCP2 is not strictly involved in (global) transcriptional repression. Recent expression profiling of hypothalamus isolated from *MeCP2*-null mice or mice overexpressing MeCP2 suggests that MeCP2 can activate transcription on the majority of its targets rather than repressing them (Chahrour et al. 2008). Although it is likely that some genes were affected indirectly in these experiments, chromatin immunoprecipitation (ChIP) showed that MeCP2 binds to promoter regions of some of the activated targets. This activation has been suggested to involve the transcriptional activator CREB-1. These results are highly surprising given the known role of MeCP2 in transcriptional repression. However, it has also been reported that the majority of

MeCP2 bound promoters have low DNA methylation and are actively expressed (Yasui et al. 2007). Moreover, a context-dependent function of MeCP2 may be supported by evidence that MeCP2-mediated transcriptional repression depends on developmental stage (OB and GJCV, manuscript in preparation), reinforcing the notion that MeCP2 is not a constitutive repressor.

Apart from the "global" MeCP2 function, its role in the regulation of specific loci has been characterized. The brain-derived neurotrophic factor (BDNF) promoter III appears to be tightly regulated by MeCP2. Studies performed on mouse and rat neuron cultures showed that MeCP2 vacates its binding site in the BDNF promoter upon membrane depolarization and calcium influx in postmitotic neurons, allowing transcription to proceed (Chen et al. 2003; Martinowich et al. 2003). Western blotting of neural lysates revealed that neuron depolarization causes MeCP2 phosphorylation and dissociation from the BDNF promoter. Once the activation signal is lost, MeCP2-mediated transcriptional repression is quickly restored. Furthermore, both studies showed that MeCP2 recruits chromatin remodeling activities to repress transcription from BDNF promoter III (Chen et al. 2003; Martinowich et al. 2003). MeCP2 can be phosphorylated at serine 421 residue by a CaMKII kinase and this phosphorylation is required for the activity-dependent BDNF transcription (Zhou et al. 2006). Moreover, phosphorylation of serine 80 is important for the association of MeCP2 with chromatin, and dephosphorylation of this residue is triggered by calcium influx in neurons (Tao et al. 2009).

Rett syndrome and neural development

Multiple model systems have been developed to elucidate the molecular functions of MeCP2 and the etiology of RTT disease. RTT is a progressive neurodevelopmental disorder involving a maturational arrest of brain development and synaptic proliferation (Amir et al. 1999; Johnston et al. 2001). The animal model that has yielded the most results is the mouse MeCP2 knockout which exhibits severe neurological symptoms resembling those of RTT patients, including limb clasping and breathing difficulties, as well as reduced brain size and body weight (Chen et al. 2001; Guy et al. 2001). The symptoms mostly occurred between 5 and 6 weeks of age, while *MeCP2*-null mice eventually died after 2 to 3 months (Chen et al. 2001; Guy et al. 2001). Conditional *MeCP2* depletion in postmitotic neurons resulted in similar, although less severe neural defects suggesting that mature neurons do not function properly in the absence of MeCP2 (Chen et al. 2001). Further functional studies showed that mice carrying an MeCP2 mutation ($MeCP2^{308}$) which results in the truncation of the C-terminal domain exhibit many RTT features such as motor dysfunction and seizures (Shahbazian et al. 2002), while overexpression of MeCP2 in transgenic mice resulted in a severe postnatal, neural phenotype (Collins et al. 2004). Altogether, the data obtained from MeCP2 manipulation in mice suggest that the mammalian central nervous system requires well-balanced MeCP2 levels for its proper function. Such a finding is of great importance for therapeutic strategies aiming to re-establish correct MeCP2 levels. It is interesting to note that mice and humans show a different sensitivity to heterozygosity for MeCP2. In both humans and mice, MeCP2 is located on the X chromosome, which due to random X inactivation causes mosaicism in affected females. MeCP2 mutations in males usually result in congenital encephalopathy and death in the first 2 years (Geerdink et al. 2002; Zeev et al. 2002). In mice, the lack of any functional MeCP2 (males $MeCP2^{-/y}$, females $MeCP2^{-/-}$) results in a RTT-like phenotype rather than encephalopathy, whereas heterozygous females ($MeCP2^{+/-}$) remain viable and fertile (Guy et al. 2001). However, a number of heterozygous *MeCP2*-null female mice displayed some RTT features at a later stage (Chen et al. 2001; Guy et al. 2001).

BDNF, an in vivo target of MeCP2 (see above), is important for synaptic plasticity (An et al. 2008; Kuczewski et al. 2009; Smart et al. 2003). To assess the contributions of improper BDNF signaling to the RTT phenotype, Chang and colleagues examined the phenotypes of conditional BNDF mutants as well as the effects of BDNF overexpression on the *MeCP2*-null mice (Chang et al. 2006). The BDNF mutants displayed many features of the RTT model mice including reduced brain size and hind limb clasping while the overexpression of BDNF in MeCP2 mutant brains resulted in increased life span and a gain of locomotor functions. This shows that correct BDNF signaling is crucial for maintaining normal brain functions and that its alteration contributes to the RTT phenotype. These and other studies (Giacometti et al. 2007; Guy et al. 2007) suggest that MeCP2-depleted neurons do not suffer irreversible damage, and that the restoration of MeCP2 levels can lead to reduction of neurological symptoms in mice. MeCP2 expression in postmitotic neurons was able to rescue the RTT-like phenotype (Luikenhuis et al. 2004), in line with earlier observations that MeCP2 depletion in postmitotic neurons induces RTT-like symptoms (Chen et al. 2001). An alternative therapeutic approach which ameliorates the RTT phenotype has also been recently described (Tropea et al. 2009). Peritoneally injected insulin-like growth factor 1 (IGF-1) peptide increased the life span of MeCP2 mutant mice and led to the improvement of their heart condition and locomotor functions. IGF-1, unlike other potential target molecules such as BDNF, is able to cross the blood–brain barrier which makes it a suitable candidate for therapy.

In comparison with mice, *Xenopus* embryos display a much earlier requirement for MeCP2. Morpholino antisense knockdown of MeCP2 and overexpression of two common RTT mutants demonstrated the requirement for MeCP2 during primary *Xenopus* neurogenesis (Stancheva et al. 2003). Embryos lacking MeCP2 and embryos overexpressing the RTT truncation mutant display altered neural patterning due to the aberrant expression of the *Hairy2a* (*hes4*) neural repressor and do not survive past the neurulation stage (Stancheva et al. 2003). The *Hairy2*a repressor is a component of the Notch/Delta signaling pathway (Davis et al. 2001), and its expression inhibits genes such as N-β-tubulin involved in neural differentiation. Balanced expression of *Hairy2a* appears to be crucial for proper neural patterning, as both MeCP2 knockdown (*Hairy2a* upregulation) and R168 MeCP2 mutant overexpression (*Hairy2a* downregulation) result in aberrant expression of neural markers (Stancheva et al. 2003). It is interesting to note that in both mice and *Xenopus*, MeCP2 takes part in neural signaling events albeit in different stages and developmental context. However, this aspect of MeCP2 function may not capture all its activities, which is discussed in the next paragraph.

MeCP2, nuclear organization, and splicing

The number of functions attributed to the MeCP2 protein has grown during the last couple of years, well beyond transcriptional repression (Chadwick and Wade 2007; Dhasarathy and Wade 2008). An MeCP2 ChIP cloning and sequencing approach revealed a number of sequences mapping to the imprinted gene cluster on chromosome 6 (Horike et al. 2005), which includes the Dlx5–Dlx6 locus. That locus was of particular interest since the maternally imprinted gene Dlx5 is involved in the pathway synthesizing γ-aminobutyric acid (GABA) which is produced less in RTT patients. Both Dlx5 and Dlx6 were found somewhat upregulated in the cortex of MeCP2-null mice, and the main reason for that seemed to be the loss of silent chromatin loops on the imprinted allele; the formation of the loops depended on the presence of MeCP2. However, a later study showed that in a number of systems, Dlx5 and Dlx6 are expressed from both alleles (Schule et al. 2007). Biallelic expression of Dlx5 was also reported in lymphoblasts originating from autistic spectrum disorder patients and healthy control individuals (Nakashima et al. 2009).

MeCP2 has also been described as a splice regulator, interacting with YB-1, a component of messenger ribonucleoprotein particles, in brain nuclear extracts (Young et al. 2005). This association proved to be sensitive to RNAse treatment, implying that the MeCP2–YB-1 complex requires RNA for its formation or stability. Microarray splicing analysis of cerebral cortex mRNA isolated from RTT mutant mice showed a number of aberrantly spliced genes, including Dlx5. The exact function of the MeCP2–YB1 interaction remains unknown; however, it is interesting to note that several links exist between proteins associated with DNA methylation-mediated repression and RNA processing (Goll et al. 2006; Jeffery and Nakielny 2004). Moreover, chicken MeCP2 (ARBP) displays high affinity for matrix/scaffold attachment regions (MARs/SARs; Weitzel et al. 1997), and the nuclear matrix is also associated with the RNA splicing machinery (Chabot et al. 1995; Wagner et al. 2003; Zeitlin et al. 1987). It is therefore tempting to speculate that MeCP2, chromatin looping, and RNA splicing are functionally linked. It is possible that an architectural role of MeCP2 unifies the diverse set of molecular functions attributed to this protein (repression, activation, splicing, organizing long-range interactions), similar to how the insulator binding protein CTCF causes activation or repression of transcription in a context and DNA methylation-dependent fashion (Bartkuhn and Renkawitz 2008; Wallace and Felsenfeld 2007).

MBD1

MBD1 is the largest MBD family member and it also represses transcription through its repression domain like other family members. It is known to act as a transcriptional repressor both in vivo and in vitro, and depending on the splicing isoform, it can bind methylated as well as unmethylated DNA (Fujita et al. 2000; Ohki et al. 1999). Like the other family members, MBD1 associates with chromatin modifiers such as the Suv39h1–HP1 complex to enhance DNA methylation-mediated transcriptional repression (Fujita et al. 2003). The functional importance of MBD1 was demonstrated in human HeLa cells, where MBD1 was shown to associate with the H3K9 methyltransferase SETDB1 (Sarraf and Stancheva 2004). During S phase the MBD1–SETDB1 complex is recruited to chromatin by the chromatin assembly factor CAF1 to establish new H3K9 methyl marks. The removal of DNA methylation disrupts the formation of MBD1–SETDB1–CAF1 interaction on the p53BP2 promoter, which leads to the loss of H3K9 methylation. The cooperation of MBD1 and CAF1 (p150) had been described before (Reese et al. 2003) as well as the involvement of CAF1 in replication-coupled histone mark deposition (Mello and Almouzni 2001). The MBD1–SETDB1 complex formation is negatively regulated by PIAS1 and PIAS3 SUMO-ligases and sumoylated MBD1 is no longer able to form the complex (Lyst et al. 2006). Since a sumoylated MBD1 can bind methylated DNA but fails to recruit SETDB1, it is possible that the balance between SETDB1 and the SUMO ligase determines the extent of MBD1-dependent repression of methylated DNA sequences. An MBD1 mouse knockout

has been obtained, but no severe developmental defects were found. *MBD1*-null mice had a normal morphology and appeared healthy, although they carried a number of minor neural defects like reduced hippocampal neurogenesis and had problems with spatial learning. Another interesting feature of this knockout was reduced genomic stability and an increase in expression of the Intracisternal-A particle retrotransposon (Zhao et al. 2003). As H3K9 methylation is involved in the silencing of genomic repetitive elements (Martens et al. 2005; Mikkelsen et al. 2007), the lack of proper H3K9 methylation may have led to reactivation of retrotransposon sequences in the *MBD1*-null mice.

MBD2

The third member of the MBD family, MBD2, is a 44-kDa protein which shares extensive sequence homology with MBD3 (Wade 2001). MBD2 is able to bind methylated CpGs in vitro and in vivo and confer DNA methylation-mediated transcriptional silencing through its repression domain (Boeke et al. 2000; Ng et al. 1999). The repression established by MBD2 is sensitive to HDAC inhibitors, in line with its association with HDAC1 and HDAC2 in the Mi-2/NuRD chromatin remodeling complex (Zhang et al. 1999). *MBD2*-null mice developed normally and remained viable and fertile, although *MBD2*-null mothers failed to nurture their pups properly (Hendrich et al. 2001). The connection between the loss of MBD2 and the observed maternal behavior is unclear. A recent study involving a triple *MeCP2*/*MBD2*/*Kaiso* mouse knockout showed no phenotype except a minor delay in neural differentiation, ruling out redundancy as an explanation for the absence of a phenotype when knocked out separately (Martin Caballero et al. 2009). MBD2, however, does play a role in helper T-cell differentiation (Hutchins et al. 2002). Normally, the induction of IL-4 during differentiation requires the GATA3 activator, however, in MBD2-null mice Gata-3 is no longer needed for IL-4 induction and as a result IL-4 is ectopically expressed in undifferentiated helper T cells (Hutchins et al. 2002). A recent study showed that MBD2 influences X-chromosome inactivation (Barr et al. 2007). Expression of the X-linked non-coding *Xist* gene inactivates the X-chromosome in *cis*, so the active X-chromosome has to silence its own *Xist* allele in order to prevent inactivation. *MBD2* null cells displayed a low level reactivation of *Xist* expression, whereas the depletion of MBD1, MeCP2, and Kaiso did not induce *Xist* upregulation. Interestingly, MBD2 antisense targeting in cultured cells showed that MBD2-depleted, lung, or colorectal cancer cell lines fail to develop tumors once injected into nude mice (Campbell et al. 2004); moreover, mouse models for intestinal tumorigenesis show a reduced tumorigenesis rate in the absence of MBD2 (Sansom et al. 2003).

MBD3

Mammalian methyl-CpG binding protein 3 (MBD3), which in contrast to amphibian MBD3 is not able to bind methylated DNA, is an essential subunit of the Mi-2/NuRD chromatin remodeling complex (Saito and Ishikawa 2002; Zhang et al. 1999). MBD2 and MBD3 associate with Mi-2/NuRD in a mutually exclusive way, thereby forming two distinct complexes (Denslow and Wade 2007; Feng and Zhang 2001; Le Guezennec et al. 2006). The association of an MBD related to mammalian MBD2/3 with NuRD has been conserved in *Drosophila*, highlighting the importance of this interaction (Marhold et al. 2004a, b). In spite of the striking sequence similarity between MBD2 and MBD3, the two proteins do not carry out redundant functions during early development. In contrast to *MBD2*-null mice which only displayed a mild maternal phenotype as discussed above, *MBD3*-null embryos are severely affected at day 8.5 and die. *MBD3*-null ES were seriously compromised in their ability to differentiate (Kaji et al. 2006; Kaji et al. 2007) as they failed to shut down the expression of undifferentiated ES cell markers such as *Oct4*, *Nanog*, and *Rex1*. In line with that observation, embryonic bodies formed from *MBD3*-null cells did not express markers like *Brachyury* and *Gata6*. Aberrant gene expression in *MBD3*-null cells is the most probable reason why cells of the inner cell mass fail to develop into late epiblast after implantation (Kaji et al. 2007). Interestingly, MTA-3, a cell type-specific subunit of an MBD3-containing Mi-2/NuRD complex, proved to be indispensable for the B lymphocyte differentiation program (Fujita et al. 2004). MBD3 knockdown in *X. laevis* embryos severely disrupted embryogenesis (Iwano et al. 2004). A morpholino antisense inhibiting the translation of both MBD3 and MBD3LF (the long form of MBD3), injected at low concentrations, induced a defective eye formation phenotype which occurred due to the misregulation of the *Pax6* gene. In contrast to mammals where both MBD2 and MBD3 interact with Mi-2/NuRD, *Xenopus* MBD3 but not MBD2 has been shown to be a subunit of the Mi-2/NuRD complex (Wade et al. 1999).

MBD4

The last member of the MBD family, MBD4, is a thymine glycosylase which acts as a DNA repair protein and targets sites of cytosine deamination (Hendrich et al. 1999). The CpG dinucleotide is under-represented in methylated genomes. The reason for that is spontaneous hydrolytic deamination of methylated cytosine which causes mCpG-TpG transitions (Bird 1980), whereas non-methylated CpG mutates to UpG. MBD4 is able to excise and repair both mutated nucleotides (Hendrich et al. 1999). In line with this

function, mutations in MBD4 have been found in various human carcinomas associated with microsatellite instability (Riccio et al. 1999). *MBD4*-null mice had a two to three times higher number of mCpG-TpG transitions showing that MBD4 acts to reduce the mCpG-TpG mutation rate (Millar et al. 2002; Wong et al. 2002). This relatively mild mutator phenotype suggests that other thymine glycosylases might carry out similar functions. In both studies, mice appeared healthy and did not show any physical abnormalities. Only when crossed with mice carrying a germline mutation in the *Apc* (adenomatous polyposis coli) gene, the *MBD4*-null animals showed accelerated tumor formation (Millar et al. 2002) or accelerated tumor progression (Wong et al. 2002). The potential function of MBD4 as a transcriptional repressor was demonstrated by a series of in vitro repression assays (Kondo et al. 2005). *LexA-MBD* fusions, when targeted to reporter constructs, showed that MBD4 is capable of silencing transcription with the same efficiency as MBD2 and MeCP2 (Kondo et al. 2005). Furthermore, this MBD4-directed repression was mediated by interactions with Sin3 and HDAC1, well-known partners of the MBD family members. Whether this transcriptional potential of MBD4 reflects its physiological role still needs to be tested. Also, a recent study in zebrafish provided evidence for a role of MBD4 in active demethylation (Rai et al. 2008). It will be interesting to see whether MBD4 can act in a similar way in higher organisms and whether other thymine glycosylases can carry out similar functions.

Kaiso (ZBTB33), ZBTB4, and ZBTB38

Besides the MBD family, other proteins such as Kaiso, ZBTB4, and ZBTB38 that are able to preferentially or specifically bind methylated DNA through zinc-finger domains have also been described (Filion et al. 2006; Prokhortchouk et al. 2001). Since its discovery, Kaiso has been attributed a number of important functions. Kaiso antisense knockdown in *X. laevis* caused a premature activation of zygotic transcription which eventually led to apoptosis and developmental arrest (Ruzov et al. 2004). This phenotype closely resembled the one induced by the DNMT1 antisense depletion (Stancheva and Meehan 2000), suggesting that DNA methylation-mediated repression mechanisms are partly responsible for repression of embryonic transcription before the mid-blastula transition. A Kaiso knockout in mice resulted in no apparent abnormalities (Prokhortchouk et al. 2006). However, when *Kaiso*-null mice were crossed with $Apc^{Min/+}$ mice susceptible for intestinal tumorigenesis, Kaiso knockouts displayed a delayed onset of tumor formation. Functional studies in mammals and amphibians (Prokhortchouk et al. 2006; Ruzov et al. 2004; Ruzov et al. 2009) suggest that the biological roles of Kaiso are not conserved. In addition to Kaiso, two other zinc-finger proteins capable of binding methylated DNA have been identified (Filion et al. 2006). ZBTB4 and ZBTB38 require only one methylated CpG for binding, unlike Kaiso which requires two. Both ZBTB4 and ZBTB38 are able to repress transcription in transfection assays, and whereas they display considerably different tissue expression profiles, both of them accumulate in the brain.

Concluding remarks

MBD proteins were initially described as interpreters of the DNA methylation mark. However, they seem to affect normal development and (patho) physiology by a wide variety of mechanisms, some of which are yet to be characterized. Developmental requirements for methyl-CpG binding protein function differ among species. Generally, MBD depletion in *Xenopus* causes more severe developmental defects than in mice. The exception is MBD3 which, in spite the lack of specific DNA-methylation binding, is indispensable for early mouse development. In humans, MeCP2 inactivation on one of the X chromosomes causes Rett syndrome in girls, whereas boys with a MeCP2 mutation are affected even more severely. In that respect, mice are less sensitive to loss of MeCP2 because a Rett-like phenotype is observed only when both MeCP2 alleles are deleted (Chen et al. 2001; Guy et al. 2001). Notwithstanding the differences observed upon MeCP2 depletion, it is interesting that both *Xenopus* and mice employ MeCP2 for gene regulation in response to neural signaling (Martinowich et al. 2003; Stancheva et al. 2003). Encouraging advances have been made in reversing Rett-like symptoms in mice (Giacometti et al. 2007; Guy et al. 2007; Luikenhuis et al. 2004; Tropea et al. 2009). A better insight in the action of MeCP2 at the molecular genomic level may unify the diverse processes MeCP2 has been attributed a function. In the near future, a greater understanding of methyl-CpG binding protein function will require integrated analyses of genome-wide binding profiles, long-range interactions in chromatin, and the regulatory role of posttranslational modifications. The rewards will be manifold, as DNA methylation makes an essential epigenetic contribution to genome integrity and function.

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