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Many paths to one goal? The proteins that recognize methylated DNA in eukaryotes

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ABSTRACT DNA methylation is an epigenetically inherited chemical modification that is associated with transcriptional silencing and is essential for mammalian development. The DNA methylation signal is read out by methyl-CpG binding proteins (MBPs) that specifically bind to methylated DNA. Three structurally divergent families of MBPs have been identified so far: the MBD family, the SRA family and a family of proteins with Zinc fingers. In this review, we describe how the distinct families of methyl-CpG binding proteins have evolved, how they each recognize and maintain the DNA methylation mark, and finally how they turn this mark into biological effect.

KEY WORDS: *DNA methylation, methyl-CpG binding proteins, MBD domain, zinc finger, SRA domain*

Introduction

DNA methylation is an epigenetically inherited chemical modification, which can occur at the adenine or cytosine bases of DNA molecules. Methylated DNA is found in various species from bacteria to humans. In bacteria, the DNA methylation system is used in combination with methylation-sensitive restriction enzymes primarily to protect the host genomes from invasion by foreign DNA. In eukaryotes, the roles of DNA methylation are much more diverse. In mammals, DNA methylation takes place mainly on the cytosine of CpG dinucleotides and plays crucial roles in X chromosome inactivation, genomic imprinting, genome stability and transcriptional silencing. DNA methylation is essential for early development (Bird, 2002, Li, 2002).

The methylated DNA signal is read out by a set of proteins which specifically recognize methylated DNA and recruit corepressor complexes to form transcriptionally silenced chromatin structures. In mammals, three structurally distinct types of Methyl-CpG binding proteins have been identified so far: the MBD family, the SRA family, and a group of proteins with Zinc fingers (Fig. 1). Here, we will review the recent data addressing the roles of these proteins in mammalian cells, and underline their similarities and differences. We will also point out interesting aspects of the evolution of methyl-CpG binding proteins in vertebrates and invertebrates.

DNA methylation: an epigenetic mark found in diverse organisms

The abundance and role of DNA methylation varies greatly between species (Colot and Rossignol, 1999). The genomes of vertebrates are consistently heavily methylated, whereas the DNA methylation patterns vary to a great extent between invertebrate species (Table 1) (Suzuki and Bird, 2008). In mammals, approximately 70% of CpG sequences are methylated (Bird, 2002). However, the CpG-rich stretches of DNA, mostly associated with gene promoters, that are called CpG-islands usually escape methylation. This rule has exceptions, and some CpG islands are known to undergo methylation during development or in somatic cells (Shen *et al.*, 2007, Weber *et al.*, 2007).

DNA methylation is generated by dedicated enzymes; their signature is a catalytic domain that is found conserved in prokaryotic and eukaryotic organisms (Goll and Bestor, 2005). Mammals have three types of DNA methyltransferases: DNMT1, DNMT2, and the closely related DNMT3a and DNMT3b. DNMT1 acts preferentially on hemi-methylated DNA and contributes to main-

Abbreviations used in this paper: BTB, broad complex, tramtrack and bric à brac; CtBP, C-terminal binding protein; DNMT, DNA methyltransferase; KBS, kaiso binding sequence; MBD, methyl-CpG-binding domain; MBP, methyl-CpG binding protein; SRA, SET and ring finger-associated.

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tain the DNA methylation patterns during cell division (Bestor, 1992, Leonhardt *et al.*, 1992). In contrast, DNMT3a and DNMT3b are *de novo* methyltransferases that act on non-methylated DNA (Okano *et al.*, 1998a, Okano *et al.*, 1999). DNMT2 has comparatively weak DNA methyltransferase activity (Okano *et al.*, 1998b), but instead methylates tRNA (Goll *et al.*, 2006). An important unresolved question is how these proteins are targeted to specific regions to generate the required patterns of DNA methylation.

Essential roles of DNA methylation in embryonic development

The levels of DNA methylation in mammals are regulated very dynamically during early development. After fertilization, both maternal and paternal genomes undergo demethylation through mechanisms that are currently unknown. Both genomes become methylated again after implantation (Li, 2002). Knockout of the *Dnmt1* gene causes a decrease of DNA methylation and results in early embryonic lethality (Li *et al.*, 1992). But is the essential function of DNMT1 to methylate DNA? This important question has been answered very recently: mice expressing a catalytically inactive mutant of DNMT1 show phenotypes identical to those of *Dnmt1*-null mice, proving that DNA methylation is indeed the essential role of DNMT1 in development (Damelin and Bestor, 2007, Takebayashi *et al.*, 2007). Combined inactivation of *Dnmt3a* and *Dnmt3b* genes blocks *de novo* methylation and also results in embryonic lethality (Okano *et al.*, 1999). In addition to its importance during development, DNA methylation is associated with various human diseases. Mutations in *Dnmt3b* cause ICF syndrome, an autosomal recessive disorder characterized by immunodeficiency, centromeric heterochromatin instability, and facial abnormalities (Hansen *et al.*, 1999, Xu *et al.*, 1999). Defects in genomic imprinting can also cause several human diseases including Beckwith–Wiedemann syndrome, Prader–Willi syndrome and Angelman syndrome, and are associated with some cancers (Feinberg, 2007).

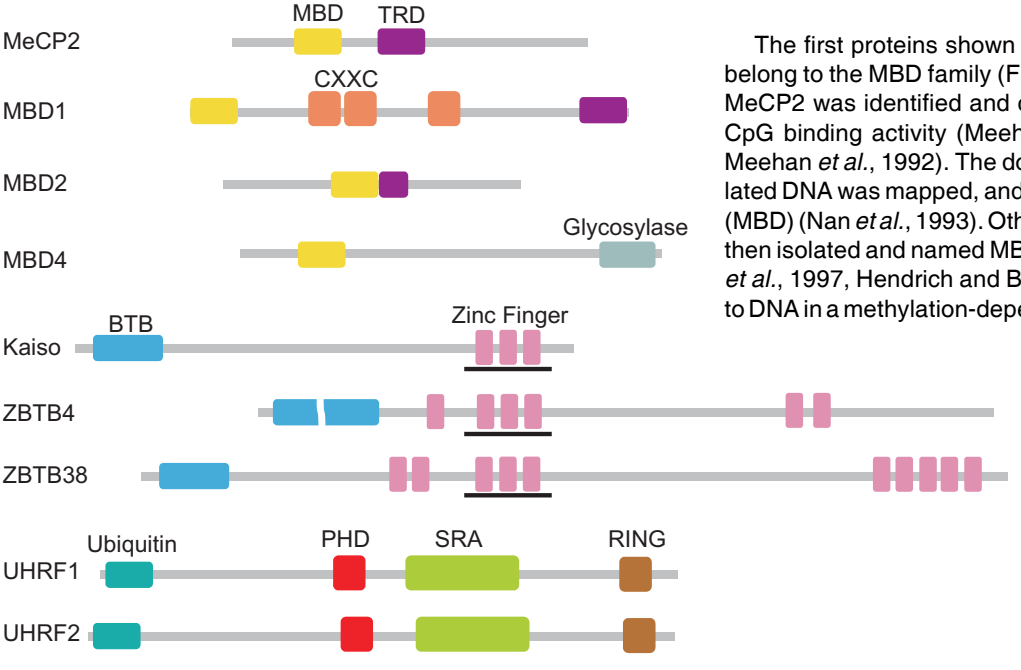

Fig. 1. The human proteins that bind methylated DNA. *Three types of domains endow proteins with the ability to recognize methylated DNA: the MBD domain, certain zinc fingers, and the SRA domain. We have represented all the human proteins known to bind methylated DNA at this time. The MBD proteins contain the MBD as well as a trans-repression domain (TRD), CXXC zinc fingers or glycosylase domain. The zinc fingers are depicted by pink rectangles, and those that confer methyl-DNA binding are underlined. The zinc finger family proteins also contain a BTB domain and up to 7 additional zinc fingers. The BTB domain of ZBTB4 is disrupted by an insertion of serine rich sequences. UHRF1 and UHRF2 contain the SRA domain, a Ubiquitin-like domain, a PHD finger and a Ring finger.*

DNA methylation is coupled to transcriptional silencing

Many studies have established that DNA methylation is associated with transcriptional silencing in mammalian cells. Two complementary phenomena are involved. First, the methylation of DNA can prevent some transcription factors from binding their target. For instance the binding of CTCF, a chromatin insulator protein, to its target sites is blocked by DNA methylation (Bell and Felsenfeld, 2000, Hark *et al.*, 2000). Second, DNA methylation creates binding sites for proteins that specifically recognize methylated DNA. These proteins, called methylated DNA binding proteins (MBPs) (Fig. 1), recruit enzymatic cofactors that modify histones, resulting in the establishment of heterochromatin, a molecular environment that inhibits gene expression. DNA methylation and histone modifications are tightly connected to establish and maintain a transcriptionally silenced chromatin environment (Fig. 2) (Fuks, 2005).

MBD-containing proteins: the original MBPs

The first proteins shown to specifically bind methylated DNA belong to the MBD family (Fig. 1). Over 15 years ago, the protein MeCP2 was identified and cloned as a component of a methyl-CpG binding activity (Meehan *et al.*, 1989, Lewis *et al.*, 1992, Meehan *et al.*, 1992). The domain responsible for binding methylated DNA was mapped, and named Methyl-CpG-binding domain (MBD) (Nan *et al.*, 1993). Other proteins containing this motif were then isolated and named MBD1, MBD2, MBD3 and MBD4 (Cross *et al.*, 1997, Hendrich and Bird, 1998). All the MBD proteins bind to DNA in a methylation-dependent manner via the MBD. The only exception is MBD3, which lacks methyl-CpG-binding activity because of amino acid changes at critical positions (Saito and Ishikawa, 2002). MBD4, which contains a thymine glycosylase domain, behaves as a DNA repair enzyme. In mammalian genomes, methylated cytosine is unstable and frequently mutated to thymine by deamination, generating T:G mismatches (Walsh and Xu, 2006). MBD4 preferentially binds to these T:G mismatches and repairs them, and its deficiency results in the enhancement of CpG mutability and tumorigenesis (Hendrich *et al.*, 1999, Millar *et al.*, 2002).

The solution structure of the MBD

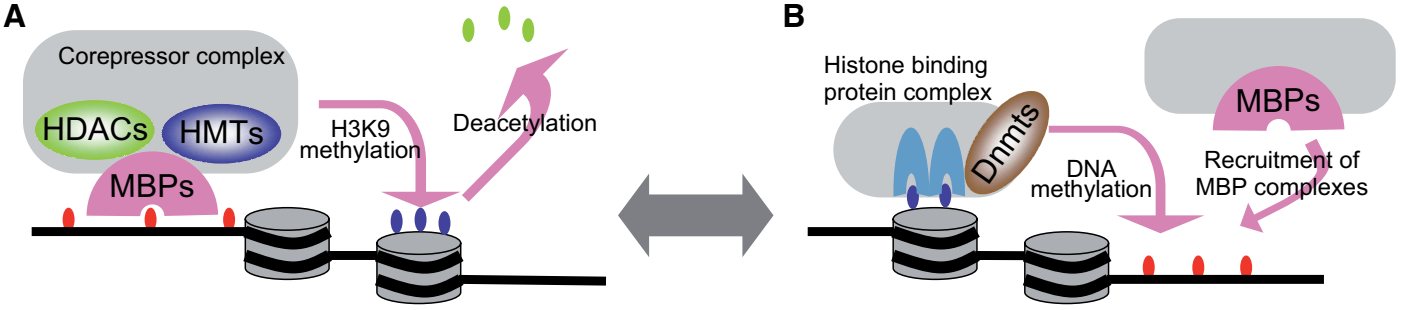

Fig. 2. Interplay between DNA methylation and histone modifications. (A) *A model for histone modifications directed by DNA methylation. Methyl-CpG binding proteins recognize methylated DNA and then recruit corepressor complexes including histone deacetylases or methyltransferases to form heterochromatin, characterized by histone hypoacetylation and H3K9 methylation.* **(B)** *A model for histone modification-directed DNA methylation. The protein complex including the histone-binding proteins recruits DNA methyltransferases to methylate DNA, resulting in the binding of MBP complexes. These two mechanisms are interconnected and contribute to establish and maintain the transcriptionally silenced chromatin structure.*

domain in complex with methylated DNA has been determined (Ohki *et al.*, 2001), and nothing in the structural model seemed to indicate that the MBD proteins would recognize DNA elements besides the methylated CpG. In other words, it seemed that the MBD proteins would bind to all methylated DNA loci irrespective of the sequence context. However, Adrian Bird and coworkers found by a modified site-selection assay that MeCP2 does have a preference for targets in which the methylated bases are surrounded by A/T rich sequences (Klose *et al.*, 2005). This opened the possibility that other MBD proteins might also bind preferentially to certain methylated targets because of the sequence flanking the methylated CpGs. Interestingly, domains other than the MBD could be involved in this sequence discrimination. For instance, MBD1 has a CXXC zinc finger-like domain that directs it towards some unmethylated CpGs (Jorgensen *et al.*, 2004). Also, the MBD proteins might be recruited to specific target regions by interaction with other proteins that bind DNA in a sequence-specific manner. For example MBD2 interacts with MIZF, which itself recognizes a consensus DNA sequence (Sekimata and Homma, 2004).

As the correlation between DNA methylation and gene silencing had long been known, it was satisfying to observe that MeCP2, MBD1 and MBD2 were transcriptional repressors (Nan *et al.*, 1997, Ng *et al.*, 1999, Ng *et al.*, 2000). An important discovery was that DNA methylation and histone modifications are strongly interconnected (Fig. 2). Indeed, the MBD proteins were found to recruit corepressors that regulate gene expression by acting on histones. These corepressors include histone deacetylases, methyltransferases, and chromatin remodeling factors. Each MBD interacts with several different coregulators, but it is not always clear if all cofactors act simultaneously in a complex, or at different times, or in different situations. For instance, MeCP2 interacts with the Sin3A/HDAC complex, the nucleosome remodeler ATRX, and also associates with an H3K9 methyltransferase activity (Jones *et al.*, 1998, Nan *et al.*, 1998, Fuks *et al.*, 2003, Nan *et al.*, 2007). MBD2 associates with the

TABLE 1

DISTRIBUTION OF DNA METHYLTRANSFERASES AND METHYL-CPG BINDING PROTEINS IN VARIOUS ORGANISMS

Organism	DNA methylation	DNMT1	DNMT3	Kaiso	ZBTB4	ZBTB38	UHRF1	UHRF2
Homo sapiens	+++[1]	Yes[2]	Yes	NP_006768	NP_065950	NP_001073881	NP_001041666	NP_690856
Mus musculus	+++	Yes	Yes	NP_064652	NP_083624	NP_780746	NP_035061	NP_659122
Gallus gallus	+++	Yes	Yes	NP_001073197	ND[3]	XP_422601	XP_418269	ENSGALP 00000024227
Xenopus tropicalis	+++	Yes	Yes	ENSXETP 00000009735	ND	ENSXETP 00000056313	ENSXETP 00000048273	ENSXETP 00000001255
Takifugu rubripes	+++	Yes	Yes	SINFRUP 00000177461	ND	SINFRUP 00000158875	SINFRUP 00000162015	SINFRUP 00000155873
Danio rerio	+++	Yes	Yes	ABI97385	ND	GENSCAN 00000008127/ XP_001339146[4]	NP_998242	
Ciona intestinalis	++	Yes	Yes	ND	ND	ND	NP_001071846	
Apis mellifera	+	Yes	Yes	ND	ND	ND	XP_001120707	
Drosophila melanogaster	+[5]	No	No	ND	ND	ND	ND	
Caenorhabditis elegans	-	No	No	ND	ND	ND	ND	
Arabidopsis thaliana	++	Yes	Yes	ND	ND	ND	NP_176091/NP_176779/NP_198771[6]	

Representative accession numbers obtained from the GenBank or Ensembl databases are indicated.

[1]+++; global methylation, ++; patchy or specific methylation, +; low level methylation, -; no methylation. [2]Yes; containing at least one protein categorized in this family. No; not found. For details, see review (Goll and Bestor, 2005). [3]ND; not detected. [4]DrZBTB38a and DrZBTB38b. [5]This methylation occurs mainly in the CpT/A sequence context. [6]*Arabidopsis* has in total 14 SRA-containing proteins including these three.

NuRD corepressor complex (Zhang *et al.*, 1999), and MBD1 with the H3-K9 histone methyltransferases SETDB1 and SUV39H1, and the heterochromatin-binding protein HP1 (Fujita *et al.*, 2003, Sarraf and Stancheva, 2004). Recent studies showed that MBD4 also acts as a transcriptional repressor by associating with the Sin3A/HDAC complex (Kondo *et al.*, 2005).

The MBD proteins: dispensable or redundant?

Given the importance of DNA methylation during embryonic development (Li *et al.*, 1992, Okano *et al.*, 1999), it was a surprise to find out that not a single gene of the MBD family is essential (Table 2). *MeCP2*, *MBD1* and *MBD2* have been knocked out in mice, and the mutant animals display only mild phenotypes (see below) (Chen *et al.*, 2001, Guy *et al.*, 2001, Hendrich *et al.*, 2001, Zhao *et al.*, 2003). Why do the phenotypes of MBD-knockout mice differ from those of Dnmt mutants? One possible explanation is that the MBD genes are functionally redundant and can compensate for the deletion of each other. However, the combined knockout of *MBD2* and *MeCP2* does not enhance either phenotype (Guy *et al.*, 2001), indicating that these two genes are not functionally overlapping. Along the same lines, the proteins MBD1, MBD2, and MeCP2 bind different loci in cultured human cells (Ballestar *et al.*, 2003, Klose *et al.*, 2005), suggesting that the proteins have different functions and may not be redundant. The generation of a triple *MeCP2*/*MBD1*/*MBD2* knockout is needed to establish if it is indeed the case.

Another explanation for the viability of MBD-knockout mice is that DNA methylation in itself might be sufficient to silence genes by inhibiting the binding of transcriptional activators. The MBD proteins could be required not for the initiation of repression, but only for its maintenance, and have only subtle effects. It is also possible that non-MBD proteins including Zinc finger or SRA-containing proteins are important in the recognition of methylated DNA during early development.

Subtle roles for MBD1 and MBD2 in the regulation of gene expression

Even though they are not essential for viability, the MBD proteins do have some important roles in other specific contexts. MBD1 is likely to be involved in the maintenance of genome stability. *MBD1* knockout mice show increased genomic instability and elevated expression of the repeated element IAP (Zhao *et al.*, 2003). Moreover, MBD1 interacts with methylpurine-DNA glycosylase (MPG), which excises damaged bases from substrate DNA, and repairs the base damage in heterochromatic region (Watanabe *et al.*, 2003). These findings raise the possibility that MBD1 links genome maintenance with transcriptionally silenced chromatin structure.

MBD2-null mice are more resistant than wild-type to intestinal tumorigenesis when crossed with the $APC^{Min/+}$ mouse, a model for human familial adenomatous polyposis (Sansom *et al.*, 2003). This indicates that MBD2 is necessary for tumor formation, presumably because it represses some methylated tumor-suppressor genes. Interestingly, MBD2 was shown to be abundant in the colon and repress the expression of exocrine pancreatic genes that are not necessary for colon function in a DNA-methylation dependent manner (Berger *et al.*, 2007). *MBD2* knockout mice also show a maternal behavioral defect (Hendrich *et al.*, 2001). The reason for this is unknown, but could be linked to the existence of many genes undergoing imprinted expression in the brain, and that might influence behavior (Davies *et al.*, 2005). MBD2 is also involved in the regulation of the *interleukin-4* gene during the differentiation of T-helper cells into Th2-helper cells (Hutchins *et al.*, 2002). In addition, several other genes are likely to be regulated by MBD2. A recent study indicated that MBD2 is involved in the repression of the methylated *Xist* gene on the active X-chromosome (Barr *et al.*, 2007).

TABLE 2

KNOCKOUT PHENOTYPES OF MBP GENES IN THE MOUSE

Gene	Phenotypes	References
MeCP2	Severe neurological symptoms that mimic Rett syndrome at approximately six weeks of age	Chen *et al.*, 2001, Guy *et al.*, 2001
MBD1	No detectable developmental defects Defect in neurogenesis, spatial learning, and long-term potentiation in the dentate gyrus of the hippocampus Reduced neuronal differentiation and increased genomic instability of neural stem cells	Zhao *et al.*, 2003
MBD2	Viable and fertile Defect in maternal behavior Genetic interaction with MBD3 Reduced tumor formation in the $APC^{Min/+}$ background	Hendrich *et al.*, 2001 Sansom *et a*l., 2003
MBD4	Viable and fertile Increased frequency of CG to TA mutations at CpG sites Accelerated tumor formation in the $APC^{Min/+}$ background	Millar *et a*l., 2002
Kaiso	No detectable developmental abnormalities No defects in neural differentiation Resistance to intestinal tumorigenesis in the $APC^{Min/+}$ background	Prokhortchouk *et al.*, 2006
UHRF1	Early gestational lethality in a similar manner to $Dnmt1^{-/-}$ embryos Reduced genomic DNA methylation and perturbation of retrotransposons and imprinted genes expression	Sharif *et al.*, 2007

Functions of the MBD proteins in the nervous system

DNA methylation is important for neurogenesis. One well-established case is that it regulates the timing of astrocyte differentiation (Kondo, 2006). For example, methylation in the CpG site within the STAT3-binding sequence located in the GFAP promoter is a critical determinant for astrocyte differentiation in the fetal brain (Takizawa *et al.*, 2001). Correspondingly, the MBD proteins appear to play important roles in the brain. MBD1 is highly expressed in the hippocampus and a subpopulation of immature cells, and *MBD1*-deficient neural stem cells show reduced neuronal differentiation (Zhao *et al.*, 2003). *MBD1* knockout ES cells or mice display decreased neurogenesis and deficits in spatial learning and dentate gyrus-specific long-term potentiation. Although the precise molecular mechanism by which MBD1 regulates these pathways is still unclear, MBD1 appears to play important roles in the epigenetic regulation in the function of Central Nervous System (CNS).

Mutations in the *MeCP2* gene are associated with Rett syndrome, an X-linked human neurological disorder that occurs almost exclusively in females (Amir *et al.*, 1999). The *MeCP2* mutant mouse also shows similar phenotypes (Chen *et al.*, 2001, Guy *et al.*, 2001), indicating that *MeCP2*-deficient mouse is a model system for human Rett syndrome. As MeCP2 functions as a transcriptional repressor, it is considered that expression levels of specific target genes of MeCP2 are impaired in the patients of

Rett syndrome or *MeCP2*-null mice. Although initial experiment by microarray analysis with wild type and *MeCP2* knockout brains failed to detect global changes of gene expression (Tudor *et al.*, 2002), recent studies identified several candidate MeCP2 target genes (Kriaucionis *et al.*, 2006; Chahrour *et al.*, 2008). Importantly, one of the target genes of MeCP2 is the neurotrophic factor *BDNF*, which is repressed by the MeCP2/Sin3A complex but activated by Ca^{2+} signaling (Chen *et al.*, 2003, Martinowich *et al.*, 2003). MeCP2 is also involved in the regulation of neuronal genes by cooperating with REST (Ballas *et al.*, 2005) and of the imprinted genes *Dlx5* and *Dlx6* (Horike *et al.*, 2005). Recently, a brain-specific micro RNA, miR-184, was shown to be regulated by MeCP2 (Nomura *et al.*, 2008). However, the mechanism of target gene regulation by MeCP2 is still not fully understood, and it could act by mechanisms other than the repression of transcriptional initiation (Bienvenu and Chelly, 2006). Indeed MeCP2 regulates splicing by interacting with the RNA-binding protein YB1 (Young *et al.*, 2005). Furthermore, a genome-wide analysis with ChIP-on-chip technology in the neuroblastoma cells identified MeCP2 binding sites exclusively in expressed genes, but not in repressed genes as was expected (Yasui *et al.*, 2007). Further analysis is required to understand the role of MeCP2 in Rett syndrome. A recent interesting discovery in the field is that the phenotype caused by lack of MeCP2 is reversible if the protein is supplied back to mutant neurons (Guy *et al.*, 2007).

Methyl-CpG binding proteins of the Zinc finger family

Three proteins are known to recognize methyl-CpG via zinc fingers: Kaiso, ZBTB4 and ZBTB38.

Kaiso was originally identified by yeast two-hybrid screening in a search for proteins that interact with the p120 catenin (Daniel and Reynolds, 1999). Kaiso contains three tandem zinc fingers at the C terminus and a BTB domain at the N terminus (Fig. 1). As p120 catenin is similar to the cell adhesion cofactors β-catenin and plakoglobin, Kaiso is speculated to be a downstream effector of β-catenin and p120 catenin signaling (van Roy and McCrea, 2005). Kaiso was also independently identified as a component of a protein complex that binds to a region of the mouse *S100A4* gene in a methylation-dependent manner (Prokhortchouk *et al.*, 2001). Whereas MBD proteins recognize a single methylated CpG, Kaiso requires two consecutive symmetrically methylated CpG sequences for optimal binding. Kaiso was also biochemically identified from HeLa cell nuclear extracts as a component of the NCoR corepressor complex including HDAC3, GPS2 and TBL1/TBLR1 (Yoon *et al.*, 2003). Kaiso directly binds to NCoR via its BTB domain; this type of interaction with corepressors involving the BTB is a common feature of BTB-zinc finger transcription factors (Collins *et al.*, 2001). Kaiso recruits the NCoR complex to the *MTA2* promoter in a DNA methylation-dependent manner, resulting in hypoacetylation and methylation at K9 of H3 at the promoter region (Yoon *et al.*, 2003). Therefore, this establishes that Kaiso is a DNA methylation-dependent transcriptional repressor of the *MTA2* gene (Fig. 3). However, as the cultured cells do not reflect the normal patterns of DNA methylation, it remains unknown whether *MTA2* is an actual target of Kaiso in normal cells and at present there is no information about methylated target genes of Kaiso in normal cells.

The human genome encodes two proteins that have Kaiso-like Zinc fingers: ZBTB4 and ZBTB38 (Filion *et al.*, 2006). Both proteins contain a BTB domain, and they are longer and more complex than Kaiso, notably because they have additional Zinc fingers (Fig. 1). These two proteins were shown, like Kaiso, to bind methylated DNA thanks to their three Kaiso-like Zinc fingers. However, in contrast to Kaiso, they can bind to a single methylated CpG sequence. ZBTB4 and ZBTB38 repress transcription of methylated promoters in a reporter assay, and localize to densely methylated pericentromeric heterochromatin regions in mouse cells in a DNA methylation-dependent manner. All these findings support the idea that ZBTB4 and ZBTB38 are bona fide methyl-CpG-binding proteins. As for Kaiso, however, their target genes in normal cells are unknown at this point.

Bimodal DNA binding activity of Kaiso and ZBTB4

In addition to binding methylated DNA, Kaiso was shown by a site-selection strategy to bind with high affinity to the DNA sequence TCCTGCNA, which is not methylated. This sequence has been named Kaiso-binding sequence or KBS (Daniel *et al.*, 2002). A KBS is present in the promoter of *matrilysin*, a target of the Wnt/beta-catenin/TCF. It is bound by Kaiso, which seems to repress its transcription *in vivo* (Fig. 3) (Spring *et al.*, 2005). In addition, Kaiso binds to the 5' beta-globin insulator that contains the KBS *in vivo* (Defossez *et al.*, 2005). ZBTB4 also can bind to the KBS, at least *in vitro*, but ZBTB38 cannot (Filion *et al.*, 2006). Of course the Kaiso-like zinc fingers of ZBTB38 may bind a different unmethylated consensus sequence that is unknown.

How do the same three Zinc fingers direct Kaiso (and ZBTB4) to methylated DNA or to unmethylated consensus sequences? Our detailed sequence analysis shows that the third finger is the least evolutionarily conserved, whereas the first and second fingers show high homology among Kaiso, ZBTB4 and ZBTB38

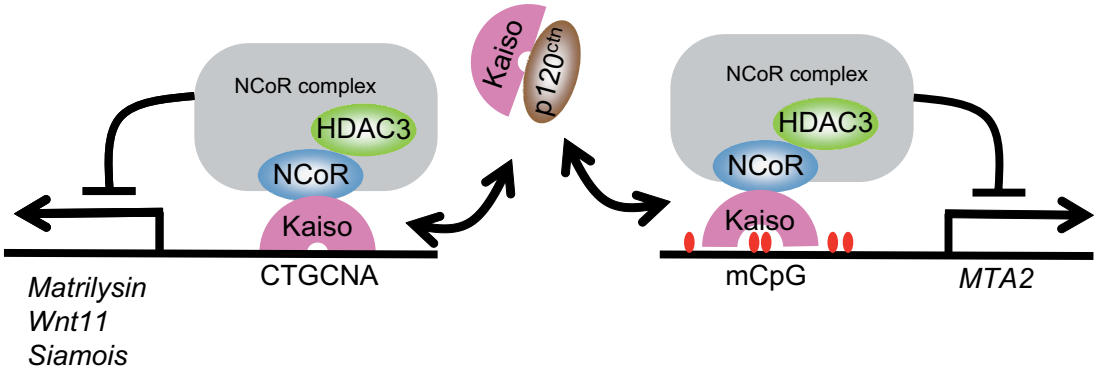

Fig. 3. Mechanisms of Kaiso-mediated transcriptional repression. *Kaiso binds to the KBS of target promoters and represses their transcription by recruiting a corepressor complex including HDAC3 and NCoR (left). Kaiso also represses methylated promoters via recruitment of the NCoR complex (right). The binding of Kaiso to these sequences is inhibited by direct interaction of p120 catenin with the zinc finger region of Kaiso.*

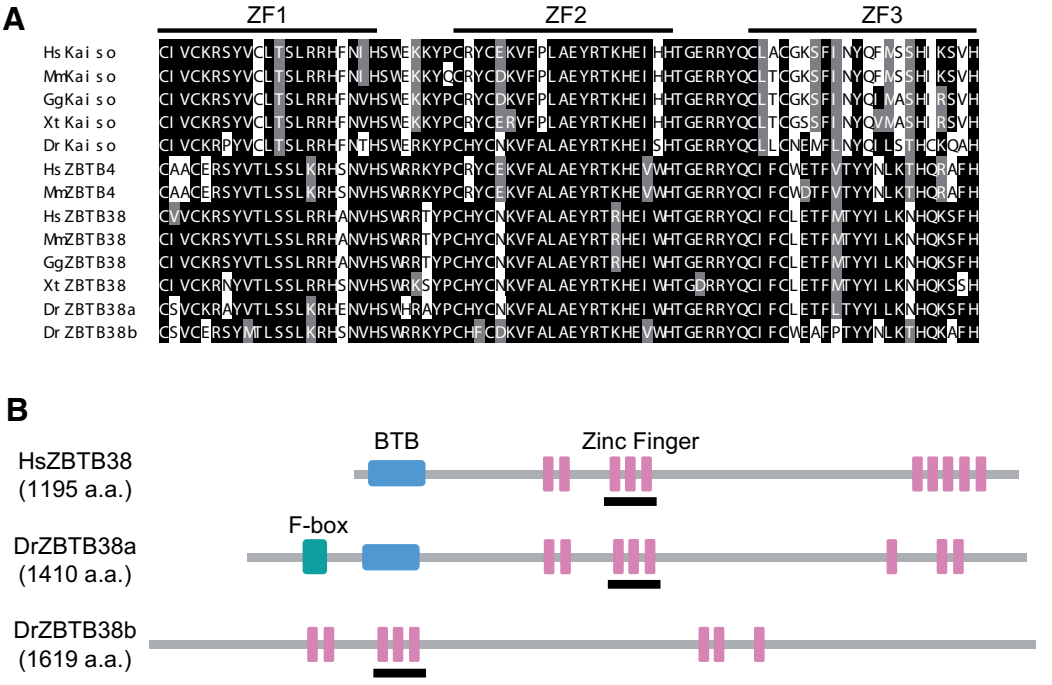

Fig. 4. Evolutionarily conserved methyl-CpG-binding zinc fingers. **(A)** *Multiple alignments of methyl-CpG binding zinc fingers of Kaiso, ZBTB4 and ZBTB38 from human* (Homo sapiens), *mouse* (Mus musculus), *chicken* (Gallus gallus), *frog (*Xenopus tropicalis*) and zebrafish* (Danio rerio). *We searched for proteins that contain three methyl-binding zinc finger motifs in the NCBI and Ensembl databases. Based on their structure and sequences, the proteins were classified as orthologs of Kaiso, ZBTB4 or ZBTB38. The alignment was performed using the Clustal W algorithm.* **(B)** *The domain structures of human (HsZBTB38) and two zebrafish ZBTB38 proteins (DrZBTB38a and DrZBTB38b). The methyl-binding zinc fingers are indicated by bold lines.*

from various species in vertebrates (Fig. 4). It suggests that these two fingers play important roles in the recognition of DNA, yet it could also be that they are involved in the interaction with partner proteins. Although Kaiso, ZBTB4, and ZBTB38 have been shown to bind to various methylated probes *in vitro* (Prokhortchouk *et al.*, 2001, Filion *et al.*, 2006), it is unknown whether their binding is influenced by the sequences flanking the methylated CpGs, as is the case of MeCP2 (Klose *et al.*, 2005).

Mechanism of transcriptional repression by Kaiso-like proteins

Kaiso represses transcription by recruiting the NCoR complex, but ZBTB4 and ZBTB38 behave differently. ZBTB38 is likely to recruit a complex containing the corepressor protein CtBP: the mouse ortholog of ZBTB38 interacts with the two closely related proteins CtBP1 and CtBP2 and recruits them to the densely methylated pericentromeric heterochromatin (Sasai *et al.*, 2005). CtBP is involved in the repression of epithelial and proapoptotic genes and knockdown of CtBP results in apoptosis in a p53-independent manner (Grooteclaes *et al.*, 2003, Zhang *et al.*, 2003). Furthermore, *CtBP1* and *CtBP2* have overlapping and unique roles during development and knockout of both genes results in embryonic lethality (Hildebrand and Soriano, 2002). CtBP associates with many transcription factors and cofactors (Chinnadurai, 2002), and ZBTB38 may be one of them. The CtBP complex includes histone deacetylases, methyltransferases and a demethylase (Shi *et al.*, 2003), and it is quite possible that ZBTB38 recruits these enzymes to repress methylated target genes.

ZBTB4 is also a transcriptional repressor, but its mode of action is unknown and likely be different from that of Kaiso or ZBTB38. Indeed, we found that the CtBP-interaction motifs found in ZBTB38 do not exist in ZBTB4. And the BTB domain, which is important for repression by Kaiso, is not required for repression by ZBTB4 (Filion *et al.*, 2006). In fact, the BTB domain of ZBTB4 is disrupted by a long serine-rich insertion and might have lost its repressive function (Perez-Torrado *et al.*, 2006). Recently, ZBTB4 was shown to associate with the Sin3/HDAC complex to repress the p21 promoter, although the repression is not dependent on binding to methylated DNA (Weber *et al.*, 2008).

Kaiso is essential for amphibian development

The *Xenopus* genome encodes a Kaiso homolog, xKaiso (Kim *et al.*, 2002). Depletion of xKaiso by morpholino oligos results in embryonic lethality with failure of gastrulation, premature activation of zygotic genes before the mid-blastula transition, and apoptosis (Kim *et al.*, 2004, Ruzov *et al.*, 2004). xKaiso has been proposed to repress several genes in the Wnt pathway that contain KBSs in their promoters (*Wnt11* (Kim *et al.*, 2004), *Siamois*, *c-Fos*, *Cyclin-D1*, and *c-Myc* (Park *et al.*, 2005)), and this could explain this effect. Interestingly, however, the xKaiso knockdown phenotypes are quite similar to those of hypomethylated embryos in which the DNA methyltransferase xDnmt1 is depleted (Stancheva and Meehan, 2000, Stancheva *et al.*, 2001). However, DNMT1 seems to have multifunctional non-enzymatic roles in development and recent report shows that xDNMT1 functions as a transcriptional repressor independent of its catalytic activity during early development (Dunican *et al.*, 2008). Nevertheless, Richard Meehan and coworkers showed very recently that the phenotypes of xKaiso-depletion were rescued by zebrafish Kaiso, which binds to methylated DNA but not to the KBS (Ruzov *et al.*, 2009b). These findings suggest that the KBS-binding function of Kaiso is not required for *Xenopus* development.

Functional redundancy among Kaiso family proteins?

Given the possible involvement of Kaiso in repressing genes in the Wnt pathway, and the importance of xKaiso in amphibians,

mouse Kaiso was expected to be important for embryonic development. However, surprisingly, the knockout of mouse *Kaiso* caused no overt phenotype (Table 2) (Prokhortchouk *et al*., 2006). The expression of proposed Kaiso target genes such as *MTA2*, *S100A4* and *Wnt11* was also unaltered in kaiso-deficient mouse tissues.

Why does *Kaiso* deficiency have different outcomes in *Xenopus* and in mouse? One possible explanation is that the roles of DNA methylation in controlling the zygotic gene program are different between mice and frogs (Prokhortchouk *et al*., 2006). This is also suggested in the case of MeCP2, which is not essential for embryonic development in mouse (Chen *et al*., 2001, Guy *et al*., 2001), but regulates expression of *Hairy2a* in *Xenopus.* Accordingly, depletion of MeCP2 in *Xenopus* results in severe defects in neurogenesis during development (Stancheva *et al*., 2003). In addition, it is also important to note the possibility that Kaiso is functionally overlapping with ZBTB4 and ZBTB38 or with MBD family proteins. A recent study indicated that Kaiso, MBD2 and MeCP2 are not redundant during embryogenesis, but have an overlapping function in neural differentiation (Martin Caballero *et al*., 2009). Given the similar DNA binding properties of Kaiso and ZBTB4, these two proteins may be functionally redundant (Filion *et al*., 2006). ZBTB4 may be the factor that rescues Kaiso deficiency in mouse, the absence of a ZBTB4 counterpart in *Xenopus* would also explain the presence of a phenotype in frogs.

Although the expression patterns of *Kaiso*, *ZBTB4* and *ZBTB38* are overlapping in several adult mouse tissues (Daniel and Reynolds, 1999, Sasai *et al*., 2005, Filion *et al*., 2006), the degree of redundancy between Kaiso, ZBTB4, and ZBTB38, is unknown and will only be clarified after the generation of mutant mice for all three genes. At present, the most likely prediction is that the genes have some overlapping, and some non-overlapping functions. Firstly, p120 catenin seems to regulate Kaiso: the Kaiso-p120 catenin interaction abolishes binding of Kaiso to DNA (either methylated or not) and inhibits its repressive action (Daniel *et al*., 2002, Kelly *et al*., 2004). ZBTB4 and ZBTB38, in contrast to Kaiso, do not interact with p120 catenin (Filion *et al*., 2006). Therefore, ZBTB4 and ZBTB38 are not likely to be involved in catenin signaling. A second difference between Kaiso and ZBTB4/ZBTB38 is their cellular distribution: in transfected mouse fibroblasts ZBTB4 and ZBTB38 primarily localize to pericentromeric heterochromatin whereas Kaiso shows a diffuse nuclear abundance pattern (Filion *et al*., 2006). Thirdly, the BTB domain of Kaiso is known to homodimerize (Daniel and Reynolds, 1999), whereas ZBTB4 and ZBTB38 cannot homodimerize via the BTB domains, but homodimerize or heterodimerize each other via zinc fingers (Kiefer *et al*., 2005, Filion *et al*., 2006). To better understand the functional differences among Kaiso family proteins, it would be useful to determine whether all three proteins bind the same target sites, or have different binding preferences.

Evolution of Kaiso family proteins: ZBTB4 is restricted to mammals

Our searches in genome databases show that Kaiso-related proteins are identifiable in vertebrates, but not in invertebrates such as *Ciona intestinalis* and in plants, even though these species contain methylated DNA (Table 1). This indicates that the Kaiso family has a vertebrate-specific role. Kaiso and ZBTB38 are found in all vertebrate genomes from zebrafish (*Danio rerio*) to humans, whereas ZBTB4 is found in mammals but not in zebrafish, *Xenopus* or chicken (*Gallus gallus*) (Table 1). This suggests the following sequence of events: *Kaiso* and *ZBTB38* arose from a common ancestor gene at some point close to the invertebrate-vertebrate transition. *ZBTB4* was then generated by duplication of the *ZBTB38* gene at some later time (Filion *et al*., 2006).

The genomes of zebrafish, medaka (*Oryzias latipes*) and stickleback (*Gasterosteus aculeatus*) contain two ZBTB38-like genes, *DrZBTB38a* and *DrZBTB38b*. DrZBTB38b lacks the BTB domain, but shows overall similarity to ZBTB38 than to ZBTB4, suggesting that this gene was generated by duplication of DrZBTB38a (Fig. 4). This duplication is specific to just some species of fish as Fugu (*Takifugu rubripes*) and *Tetraodon nigroviridis* contain only one *ZBTB38* gene, like *Xenopus tropicalis* and *Xenopus laevis*. Interestingly, this is reminiscent of *MBD3*, for which two related genes are found in zebrafish and Fugu, but only one in mammals (Hendrich and Tweedie, 2003). Because we do not precisely know which amino-acids are important for binding DNA, it is not yet possible to predict if the Kaiso-like proteins in lower vertebrates bind the KBS, or methylated DNA, or both, or neither. As for Kaiso, it seems that the methyl-binding activity is evolutionarily conserved, whereas the KBS-binding activity varies between species (Ruzov *et al*., 2009).

SRA proteins: essential proteins for the maintenance of DNA methylation

The third class of methyl-CpG binding factors are UHRF1 (also called Np95, and ICBP90) and the related protein UHRF2 (also called NIRF). In these proteins the SRA domain (SET and Ring finger-associated) is responsible for methyl-CpG binding. They also contain a ubiquitin-like domain, a Ring finger motif and a PHD finger (Fig. 1). UHRF1 was initially identified as a protein that binds to a CCAAT box in the promoter of the *topoisomerase IIα* gene (Hopfner *et al*., 2000). However, UHRF1 and UHRF2 were subsequently shown to bind with higher affinity to methylated DNA than to the CCAAT box (Unoki *et al*., 2004). UHRF1 is involved in the regulation of the cell cycle and DNA damage control (Bonapace *et al*., 2002, Muto *et al*., 2002), and regulates cell proliferation by repressing methylated tumor suppressor genes via recruitment of the HDAC complex to the promoters (Unoki *et al*., 2004). UHRF1 colocalizes with replication foci in mid-to late S phase and is required for proper heterochromatin formation in mammalian cells (Papait *et al*., 2007). Since the Ring finger of UHRF1 functions as an E3 ubiquitin ligase for histone H3 (Citterio *et al*., 2004), UHRF1 was supposed to translate DNA methylation into histone modifications.

Recently, two groups clearly demonstrated that UHRF1 mediates epigenetic inheritance of DNA methylation (Bostick *et al*., 2007, Sharif *et al*., 2007). UHRF1 associates with DNMT1 and PCNA at replicating heterochromatic regions. As UHRF1 has preferential binding affinity for hemi-methylated DNA over fully methylated DNA (Bostick *et al*., 2007), DNMT1 is specifically recruited to hemi-methylated sites by UHRF1 during DNA replication. UHRF1 knockout mice die during early embryogenesis just like *Dnmt1* mutants (Table 2) (Sharif *et al*., 2007). Furthermore, in UHRF1-deficient mouse and ES cells, methylation of imprinted genes and satellite repeats are decreased. All in all, UHRF1 is

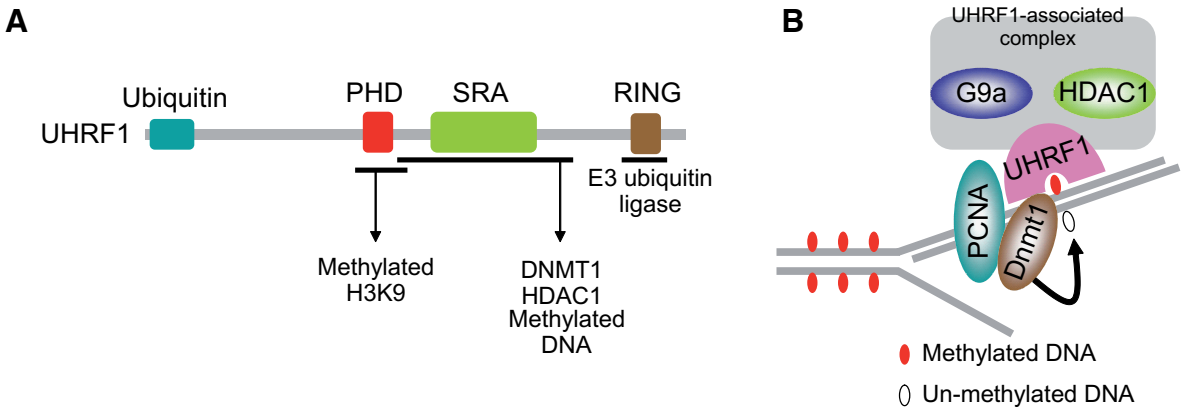

Fig. 5. Functions of UHRF1 during DNA replication. (A) *Schematic representation of UHRF1. UHRF1 binds to methylated DNA as well as DNMT1 and HDAC1 via the region including the SRA domain. The PHD finger binds to methylated K9 of H3 and the Ring finger functions as an E3 ubiquitin ligase.* **(B)** *A model for the maintenance of DNA methylation by the UHRF1-DNMT1 complex during DNA replication. UHRF1 binds to hemi-methylated DNA at the replication foci and recruits DNMT1 to maintain the DNA methylation status. UHRF1 is also likely to ensure the heritability of histone modification by recruiting the G9a-containing complex.*

considered very important for the targeting of DNMT1 to hemi-methylated DNA, in order to ensure that DNA becomes re-methylated after replication (Fig. 5). In addition, since the UHRF1 complex contains G9a histone methyltransferase and the PHD finger of UHRF1 binds to methylated K9 of H3 (Sharif *et al.*, 2007, Karagianni *et al.*, 2008), UHRF1 may be important for the maintenance of histone marks as well. Furthermore, the crystal structure of the SRA domain of UHRF1 bound to hemi-methylated DNA revealed that the SRA domain recognizes methylated DNA by flipping methyl-cytosine out of the DNA helix (Arita *et al.*, 2008; Avvakumov *et al.*, 2008; Hashimoto *et al.*, 2008), which is quite distinct from the recognition of symmetrically methylated DNA by the MBD domain (Ho *et al.*, 2008). These studies answered the important question of why DNMT1 has an innate capacity to recognize hemimethylated DNA (Jeltsch, 2006). However, it remains unknown whether UHRF1 is required for maintaining DNA methylation over the whole genome, or just at some specific regions. Also, does the related protein UHRF2 play a similar role?

At any rate, the situation of UHRF1 is reminiscent of MBD1, which associates with the p150 subunit of CAF-1 and PCNA during S-phase and maintain H3-K9 methylation via recruitment of the histone methyltransferase SETDB1 to methylated DNA (Sarraf and Stancheva, 2004). It would be interesting to see whether the MBD1 complex acts simultaneously with the SRA proteins or whether it functions independently. Other unknown factors might be also involved in maintaining DNA methylation. For instance, the SWI/SNF chromatin remodeling factor Lsh, and CGBP, a protein that binds non-methylated CpG, are known to be required for global methylation of the genome (Dennis *et al.*, 2001, Carlone *et al.*, 2005). These findings suggest that the initiation, recognition, and maintenance of DNA methylation is complex, and that additional players may remain to be discovered.

Roles of methyl-CpG binding proteins in invertebrates and in plants

MBD genes exist not only in vertebrates, but also in invertebrates and in plants, and *MBD2/MBD3* is thought to be the ancestor of the MBD genes (Hendrich and Tweedie, 2003). *Drosophila* contains only one MBD protein (dMBD2/3) and one DNA methyltransferase (dDnmt2) and its genome is methylated at CpT/A sequences, which are recognized by dMBD2/3 (Kunert *et al.*, 2003, Marhold *et al.*, 2004). These two proteins are not essential for development: flies lacking these genes are viable and fertile. *Caenorhabditis elegans* has an MBD-like gene, even though its genome is unmethylated. Interestingly, the MBD-like protein in *C. elegans* plays important roles in development, suggesting that CeMBD has functions other than binding methylated DNA (Gutierrez and Sommer, 2004). Similarly, mammalian MBD3 does not bind methylated DNA, yet it is essential for development and plays important roles in pluripotency of ES cells (Hendrich *et al.*, 2001, Kaji *et al.*, 2006). The genome of *Arabidopsis thaliana* contains 13 genes for MBD-containing proteins (Zemach and Grafi, 2007), of which at least three can bind methylated DNA. Some, like AtMBD9 and AtMBD11, are not functional as methyl-CpG binding proteins but are nevertheless important for plant development. Furthermore, vertebrate and invertebrate genomes contain a number of MBD-like domains, called TAM (TIP5, ARBP, MBD), most of which are predicted to be non-functional for binding to methyl-CpG (Hendrich and Tweedie, 2003). It seems that these MBD-like domains have lost methyl-binding capacity as they were gaining new functions during evolution.

Like the MBD proteins, the SRA proteins are found in various invertebrate genomes including that of the social insect honeybee (*Apis mellifera*), as well as in plant genomes, suggesting that they may regulate DNA methylation in these species (Table 1). At least 14 SRA-containing proteins are found in *Arabidopsis*, and other plants also contain many SRA proteins. Like mammalian SRA-containing proteins, the *Arabidopsis* SRA proteins Vim1 and KRYPTONITE (KYP) bind to methylated DNA via the SRA domain (Johnson *et al.*, 2007, Woo *et al.*, 2007). The high number of SRA proteins in plants might result from the complexity of DNA methylation in plants where not only CpG, but also CpHpG (where H = A, T or C) and CpHpH sequences can be methylated. Indeed, Vim1 can bind to methylated CpHpG sequence as well as methylated CpG, whereas KYP binds to methylated CpG, CpHpG and CpHpH sequences. Although it remains to be tested whether this binding preference is also shown on the hemi-methylated DNA,

the plant SRA proteins might have evolved the ability to bind to different methylated sequences. Alternatively, it is possible that some of the plant SRA proteins are not methyl-CpG binding proteins, but have important functions in histone modifications or other types of chromatin regulation. Indeed, some SRA proteins in plants have a SET domain that may function as a histone methyltransferase (Baumbusch *et al.*, 2001).

Interestingly, the honeybee contains methylated DNA in the context of CpG sequence, as well as MBD proteins, SRA proteins, and maintenance and *de novo* methyltransferases (Wang *et al.*, 2006). This is in sharp contrast to *Drosophila* and *C. elegans* that lack *de novo* and maintenance DNA methyltransferases and SRA proteins and have lower levels of CpT/A methylation or no DNA methylation, respectively (Table 1). These findings imply that *de novo*/maintenance DNMTs and SRA proteins may have co-evolved to ensure the faithful maintenance of DNA methylation.

Are there additional methyl-CpG binding proteins?

Considering that DNA methylation has a wide variety of roles in mammalian genomes, DNA methylation might be interpreted by not just three families of proteins. The zinc finger is one of the most common motifs in the mammalian genome. Although the Kaiso-like zinc finger motif is‘found in only three proteins Kaiso, ZBTB4 and ZBTB38, it is conceivable that additional proteins might also have the ability to bind to methyl-CpG via zinc fingers. Indeed, an artificial zinc finger protein has been shown to bind to methylated DNA (Choo, 1998). Although it is difficult to identify such proteins at the moment, the proteins that localize to densely methylated pericentromeric heterochromatin region, like MBD proteins or Kaiso-like proteins (Hendrich and Bird, 1998, Filion *et al.*, 2006), could be possible candidates. It is also possible that novel methyl-CpG binding proteins (domains) might be discovered, maybe through biochemical approaches, or by structural analyses.

Concluding remarks

After the discovery that DNA methylation and transcriptional repression are correlated, numerous factors including MBPs, histone modification enzymes, chromatin remodeling factors, DNA methyltransferases and histone binding proteins have been identified and shown to link DNA methylation and transcriptional silencing. However, we are still far from a complete understanding of this complex system and there are many issues to be uncovered. How are these proteins targeted to specific regions? How is the DNA methylation mark erased and re-established during development? The recent advances in genome-wide analysis techniques, the availability of complete genome sequences, and of genetically engineered mice and cell lines will be helpful to overcome these issues. Given the involvement of DNA methylation in development, cancer and human diseases, a greater understanding of the molecular mechanisms of DNA methylation, and of the roles of MBPs, is of great importance for the development of future clinical applications.

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References

AMIR, R.E., VAN DEN VEYVER, I.B., WAN, M., TRAN, C.Q., FRANCKE, U. and ZOGHBI, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 23: 185-188.

ARITA, K., ARIYOSHI, M., TOCHIO, H., NAKAMURA, Y. and SHIRAKAWA, M. (2008). Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. *Nature* 455: 818-21.

AVVAKUMOV, G.V., WALKER, J.R., XUE, S., LI, Y., DUAN, S., BRONNER, C., ARROWSMITH, C.H. and DHE-PAGANON, S. (2008). Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. *Nature* 455: 822-5.

BALLAS, N., GRUNSEICH, C., LU, D.D., SPEH, J.C. and MANDEL, G. (2005). REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* 121: 645-657.

BALLESTAR, E., PAZ, M.F., VALLE, L., WEI, S., FRAGA, M.F., ESPADA, J., CIGUDOSA, J.C., HUANG, T.H. and ESTELLER, M. (2003). Methyl-CpG binding proteins identify novel sites of epigenetic inactivation in human cancer. *EMBO J* 22: 6335-6345.

BARR, H., HERMANN, A., BERGER, J., TSAI, H.H., ADIE, K., PROKHORTCHOUK, A., HENDRICH, B. and BIRD, A. (2007). Mbd2 contributes to DNA methylation-directed repression of the Xist gene. *Mol Cell Biol* 27: 3750-3757.

BAUMBUSCH, L.O., THORSTENSEN, T., KRAUSS, V., FISCHER, A., NAUMANN, K., ASSALKHOU, R., SCHULZ, I., REUTER, G. and AALEN, R.B. (2001). The *Arabidopsis thaliana* genome contains at least 29 active genes encoding SET domain proteins that can be assigned to four evolutionarily conserved classes. *Nucleic Acids Res* 29: 4319-4333.

BELL, A.C. and FELSENFELD, G. (2000). Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. *Nature* 405: 482-485.

BERGER, J., SANSOM, O., CLARKE, A. and BIRD, A. (2007). MBD2 is required for correct spatial gene expression in the gut. *Mol Cell Biol* 27: 4049-4057.

BESTOR, T.H. (1992). Activation of mammalian DNA methyltransferase by cleavage of a Zn binding regulatory domain. *EMBO J* 11: 2611-2617.

BIENVENU, T. and CHELLY, J. (2006). Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized. *Nat Rev Genet* 7: 415-426.

BIRD, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev* 16: 6-21.

BONAPACE, I.M., LATELLA, L., PAPAIT, R., NICASSIO, F., SACCO, A., MUTO, M., CRESCENZI, M. and DI FIORE, P.P. (2002). Np95 is regulated by E1A during mitotic reactivation of terminally differentiated cells and is essential for S phase entry. *J Cell Biol* 157: 909-914.

BOSTICK, M., KIM, J.K., ESTEVE, P.O., CLARK, A., PRADHAN, S. and JACOBSEN, S.E. (2007). UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* 317: 1760-1764.

CARLONE, D.L., LEE, J.H., YOUNG, S.R., DOBROTA, E., BUTLER, J.S., RUIZ, J. and SKALNIK, D.G. (2005). Reduced genomic cytosine methylation and defective cellular differentiation in embryonic stem cells lacking CpG binding protein. *Mol Cell Biol* 25: 4881-4891.

CHAHROUR, M., JUNG, S.Y., SHAW, C., ZHOU, X., WONG, S.T., QIN, J. and ZOGHBI, H.Y. (2008). MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* 320: 1224-9.

CHEN, R.Z., AKBARIAN, S., TUDOR, M. and 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, W.G., CHANG, Q., LIN, Y., MEISSNER, A., WEST, A.E., GRIFFITH, E.C., JAENISCH, R. and GREENBERG, M.E. (2003). Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* 302: 885-889.

CHINNADURAI, G. (2002). CtBP, an unconventional transcriptional corepressor in development and oncogenesis. *Mol Cell* 9: 213-224.

CHOO, Y. (1998). Recognition of DNA methylation by zinc fingers. *Nat Struct Biol* 5: 264-265.

CITTERIO, E., PAPAIT, R., NICASSIO, F., VECCHI, M., GOMIERO, P., MANTOVANI, R., DI FIORE, P.P. and BONAPACE, I.M. (2004). Np95 is a histone-binding protein endowed with ubiquitin ligase activity. *Mol Cell Biol* 24: 2526-2535.

COLLINS, T., STONE, J.R. and WILLIAMS, A.J. (2001). All in the family: the BTB/POZ, KRAB, and SCAN domains. *Mol Cell Biol* 21: 3609-3615.

COLOT, V. and ROSSIGNOL, J.L. (1999). Eukaryotic DNA methylation as an evolutionary device. *Bioessays* 21: 402-411.

CROSS, S.H., MEEHAN, R.R., NAN, X. and BIRD, A. (1997). A component of the transcriptional repressor MeCP1 shares a motif with DNA methyltransferase and HRX proteins. *Nat Genet* 16: 256-259.

DAMELIN, M. and BESTOR, T.H. (2007). Biological functions of DNA methyltransferase 1 require its methyltransferase activity. *Mol Cell Biol* 27: 3891-3899.

DANIEL, J.M. and REYNOLDS, A.B. (1999). The catenin p120(ctn) interacts with Kaiso, a novel BTB/POZ domain zinc finger transcription factor. *Mol Cell Biol* 19: 3614-3623.

DANIEL, J.M., SPRING, C.M., CRAWFORD, H.C., REYNOLDS, A.B. and BAIG, A. (2002). The p120(ctn)-binding partner Kaiso is a bi-modal DNA-binding protein that recognizes both a sequence-specific consensus and methylated CpG dinucleotides. *Nucleic Acids Res* 30: 2911-2919.

DAVIES, W., ISLES, A.R. and WILKINSON, L.S. (2005). Imprinted gene expression in the brain. *Neurosci Biobehav Rev* 29: 421-430.

DEFOSSEZ, P.A., KELLY, K.F., FILION, G.J., PEREZ-TORRADO, R., MAGDINIER, F., MENONI, H., NORDGAARD, C.L., DANIEL, J.M. and GILSON, E. (2005). The human enhancer blocker CTC-binding factor interacts with the transcription factor Kaiso. *J Biol Chem* 280: 43017-43023.

DENNIS, K., FAN, T., GEIMAN, T., YAN, Q. and MUEGGE, K. (2001). Lsh, a member of the SNF2 family, is required for genome-wide methylation. *Genes Dev* 15: 2940-2944.

DUNICAN, D.S., RUZOV, A., HACKETT, J.A. and MEEHAN, R.R. (2008). xDnmt1 regulates transcriptional silencing in pre-MBT *Xenopus* embryos independently of its catalytic function. *Development* 135: 1295-1302.

FEINBERG, A.P. (2007). Phenotypic plasticity and the epigenetics of human disease. *Nature* 447: 433-440.

FILION, G.J., ZHENILO, S., SALOZHIN, S., YAMADA, D., PROKHORTCHOUK, E. and DEFOSSEZ, P.A. (2006). A family of human zinc finger proteins that bind methylated DNA and repress transcription. *Mol Cell Biol* 26: 169-181.

FUJITA, N., WATANABE, S., ICHIMURA, T., TSURUZOE, S., SHINKAI, Y., TACHIBANA, M., CHIBA, T. and NAKAO, M. (2003). Methyl-CpG binding domain 1 (MBD1) interacts with the Suv39h1-HP1 heterochromatic complex for DNA methylation-based transcriptional repression. *J Biol Chem* 278: 24132-24138.

FUKS, F. (2005). DNA methylation and histone modifications: teaming up to silence genes. *Curr Opin Genet Dev* 15: 490-495.

FUKS, F., HURD, P.J., WOLF, D., NAN, X., BIRD, A.P. and KOUZARIDES, T. (2003). The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J Biol Chem* 278: 4035-4040.

GOLL, M.G. and BESTOR, T.H. (2005). Eukaryotic cytosine methyltransferases. *Annu Rev Biochem* 74: 481-514.

GOLL, M.G., KIRPEKAR, F., MAGGERT, K.A., YODER, J.A., HSIEH, C.L., ZHANG, X., GOLIC, K.G., JACOBSEN, S.E. and BESTOR, T.H. (2006). Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. *Science* 311: 395-398.

GROOTECLAES, M., DEVERAUX, Q., HILDEBRAND, J., ZHANG, Q., GOODMAN, R.H. and FRISCH, S.M. (2003). C-terminal-binding protein corepresses epithelial and proapoptotic gene expression programs. *Proc Natl Acad Sci U S A* 100: 4568-4573.

GUTIERREZ, A. and SOMMER, R.J. (2004). Evolution of dnmt-2 and mbd-2-like genes in the free-living nematodes Pristionchus pacificus, Caenorhabditis elegans and Caenorhabditis briggsae. *Nucleic Acids Res* 32: 6388-6396.

GUY, J., GAN, J., SELFRIDGE, J., COBB, S. and BIRD, A. (2007). Reversal of neurological defects in a mouse model of Rett syndrome. *Science* 315: 1143-1147.

GUY, J., HENDRICH, B., HOLMES, M., MARTIN, J.E. and BIRD, A. (2001). A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat Genet* 27: 322-326.

HANSEN, R.S., WIJMENGA, C., LUO, P., STANEK, A.M., CANFIELD, T.K., WEEMAES, C.M. and GARTLER, S.M. (1999). The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. *Proc Natl Acad Sci U S A* 96: 14412-14417.

HARK, A.T., SCHOENHERR, C.J., KATZ, D.J., INGRAM, R.S., LEVORSE, J.M. and TILGHMAN, S.M. (2000). CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature* 405: 486-489.

HASHIMOTO, H., HORTON, J.R., ZHANG, X., BOSTICK, M., JACOBSEN, S.E. and CHENG, X. (2008). The SRA domain of UHRF1 flips 5-methylcytosine out of the DNA helix. *Nature* 455: 826-9.

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

HENDRICH, B., GUY, J., RAMSAHOYE, B., WILSON, V.A. and BIRD, A. (2001). Closely related proteins MBD2 and MBD3 play distinctive but interacting roles in mouse development. *Genes Dev* 15: 710-723.

HENDRICH, B., HARDELAND, U., NG, H.H., JIRICNY, J. and BIRD, A. (1999). The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. *Nature* 401: 301-304.

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

HILDEBRAND, J.D. and SORIANO, P. (2002). Overlapping and unique roles for C-terminal binding protein 1 (CtBP1) and CtBP2 during mouse development. *Mol Cell Biol* 22: 5296-5307.

HO, K.L., MCNAE, I.W., SCHMIEDEBERG, L., KLOSE, R.J., BIRD, A.P. and WALKINSHAW, M.D. (2008). MeCP2 binding to DNA depends upon hydration at methyl-CpG. *Mol Cell* 29: 525-31.

HOPFNER, R., MOUSLI, M., JELTSCH, J.M., VOULGARIS, A., LUTZ, Y., MARIN, C., BELLOCQ, J.P., OUDET, P. and BRONNER, C. (2000). ICBP90, a novel human CCAAT binding protein, involved in the regulation of topoisomerase IIalpha expression. *Cancer Res* 60: 121-128.

HORIKE, S., CAI, S., MIYANO, M., CHENG, J.F. and KOHWI-SHIGEMATSU, T. (2005). Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. *Nat Genet* 37: 31-40.

HUTCHINS, A.S., MULLEN, A.C., LEE, H.W., SYKES, K.J., HIGH, F.A., HENDRICH, B.D., BIRD, A.P. and REINER, S.L. (2002). Gene silencing quantitatively controls the function of a developmental trans-activator. *Mol Cell* 10: 81-91.

JELTSCH, A. (2006). On the enzymatic properties of Dnmt1: specificity, processivity, mechanism of linear diffusion and allosteric regulation of the enzyme. *Epigenetics* 1: 63-66.

JOHNSON, L.M., BOSTICK, M., ZHANG, X., KRAFT, E., HENDERSON, I., CALLIS, J. and JACOBSEN, S.E. (2007). The SRA methyl-cytosine-binding domain links DNA and histone methylation. *Curr Biol* 17: 379-384.

JONES, P.L., VEENSTRA, G.J., WADE, P.A., VERMAAK, D., KASS, S.U., LANDSBERGER, N., STROUBOULIS, J. and WOLFFE, A.P. (1998). Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet* 19: 187-191.

JORGENSEN, H.F., BEN-PORATH, I. and BIRD, A.P. (2004). Mbd1 is recruited to both methylated and nonmethylated CpGs via distinct DNA binding domains. *Mol Cell Biol* 24: 3387-3395.

KAJI, K., CABALLERO, I.M., MACLEOD, R., NICHOLS, J., WILSON, V.A. and HENDRICH, B. (2006). The NuRD component Mbd3 is required for pluripotency of embryonic stem cells. *Nat Cell Biol* 8: 285-292.

KARAGIANNI, P., AMAZIT, L., QIN, J. and WONG, J. (2008). ICBP90, a novel methyl K9 H3 binding protein linking protein ubiquitination with heterochromatin formation. *Mol Cell Biol* 28: 705-717.

KELLY, K.F., SPRING, C.M., OTCHERE, A.A. and DANIEL, J.M. (2004). NLS-dependent nuclear localization of p120ctn is necessary to relieve Kaiso-mediated transcriptional repression. *J Cell Sci* 117: 2675-2686.

KIEFER, H., CHATAIL-HERMITTE, F., RAVASSARD, P., BAYARD, E., BRUNET, I. and MALLET, J. (2005). ZENON, a novel POZ Kruppel-like DNA binding protein associated with differentiation and/or survival of late postmitotic neurons. *Mol Cell Biol* 25: 1713-1729.

KIM, S.W., FANG, X., JI, H., PAULSON, A.F., DANIEL, J.M., CIESIOLKA, M., VAN ROY, F. and MCCREA, P.D. (2002). Isolation and characterization of XKaiso,

a transcriptional repressor that associates with the catenin Xp120(ctn) in *Xenopus laevis*. *J Biol Chem* 277: 8202-8208.

KIM, S.W., PARK, J.I., SPRING, C.M., SATER, A.K., JI, H., OTCHERE, A.A., DANIEL, J.M. and MCCREA, P.D. (2004). Non-canonical Wnt signals are modulated by the Kaiso transcriptional repressor and p120-catenin. *Nat Cell Biol* 6: 1212-1220.

KLOSE, R.J., SARRAF, S.A., SCHMIEDEBERG, L., MCDERMOTT, S.M., STANCHEVA, I. and BIRD, A.P. (2005). DNA binding selectivity of MeCP2 due to a requirement for A/T sequences adjacent to methyl-CpG. *Mol Cell* 19: 667-678.

KONDO, E., GU, Z., HORII, A. and FUKUSHIGE, S. (2005). The thymine DNA glycosylase MBD4 represses transcription and is associated with methylated p16(INK4a) and hMLH1 genes. *Mol Cell Biol* 25: 4388-4396.

KONDO, T. (2006). Epigenetic alchemy for cell fate conversion. *Curr Opin Genet Dev* 16: 502-50□7.

KRIAUCIONIS, S., PATERSON, A., CURTIS, J., GUY, J., MACLEOD, N. and BIRD, A. (2006). Gene expression analysis exposes mitochondrial abnormalities in a mouse model of Rett syndrome. *Mol Cell Biol* 26: 5033-5042.

KUNERT, N., MARHOLD, J., STANKE, J., STACH, D. and LYKO, F. (2003). A Dnmt2-like protein mediates DNA methylation in *Drosophila*. *Development* 130: 5083-5090.

LEONHARDT, H., PAGE, A.W., WEIER, H.U. and BESTOR, T.H. (1992). A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell* 71: 865-873.

LEWIS, J.D., MEEHAN, R.R., HENZEL, W.J., MAURER-FOGY, I., JEPPESEN, P., KLEIN, F. and BIRD, A. (1992). Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* 69: 905-914.

LI, E. (2002). Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* 3: 662-673.

LI, E., BESTOR, T.H. and JAENISCH, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 69: 915-926.

MARHOLD, J., KRAMER, K., KREMMER, E. and LYKO, F. (2004). The *Drosophila* MBD2/3 protein mediates interactions between the MI-2 chromatin complex and CpT/A-methylated DNA. *Development* 131: 6033-6039.

MARTIN CABALLERO, I., HANSEN, J., LEAFORD, D., POLLARD, S. and HENDRICH, B.D. (2009). The methyl-CpG binding proteins Mecp2, Mbd2 and Kaiso are dispensable for mouse embryogenesis, but play a redundant function in neural differentiation. *PLoS ONE* 4: e4315.

MARTINOWICH, K., HATTORI, D., WU, H., FOUSE, S., HE, F., HU, Y., FAN, G. and SUN, Y.E. (2003). DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* 302: 890-893.

MEEHAN, R.R., LEWIS, J.D. and BIRD, A.P. (1992). Characterization of MeCP2, a vertebrate DNA binding protein with affinity for methylated DNA. *Nucleic Acids Res* 20: 5085-5092.

MEEHAN, R.R., LEWIS, J.D., MCKAY, S., KLEINER, E.L. and BIRD, A.P. (1989). Identification of a mammalian protein that binds specifically to DNA containing methylated CpGs. *Cell* 58: 499-507.

MILLAR, C.B., GUY, J., SANSOM, O.J., SELFRIDGE, J., MACDOUGALL, E., HENDRICH, B., KEIGHTLEY, P.D., BISHOP, S.M., CLARKE, A.R. and BIRD, A. (2002). Enhanced CpG mutability and tumorigenesis in MBD4-deficient mice. *Science* 297: 403-405.

MUTO, M., KANARI, Y., KUBO, E., TAKABE, T., KURIHARA, T., FUJIMORI, A. and TATSUMI, K. (2002). Targeted disruption of Np95 gene renders murine embryonic stem cells hypersensitive to DNA damaging agents and DNA replication blocks. *J Biol Chem* 277: 34549-34555.

NAN, X., CAMPOY, F.J. and BIRD, A. (1997). MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell* 88: 471-481.

NAN, X., HOU, J., MACLEAN, A., NASIR, J., LAFUENTE, M.J., SHU, X., KRIAUCIONIS, S. and BIRD, A. (2007). Interaction between chromatin proteins MECP2 and ATRX is disrupted by mutations that cause inherited mental retardation. *Proc Natl Acad Sci U S A* 104: 2709-2714.

NAN, X., MEEHAN, R.R. and BIRD, A. (1993). Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. *Nucleic Acids Res* 21: 4886-4892.

NAN, X., NG, H.H., JOHNSON, C.A., LAHERTY, C.D., TURNER, B.M., EISENMAN, R.N. and BIRD, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* 393: 386-389.

NG, H.H., JEPPESEN, P. and BIRD, A. (2000). Active repression of methylated genes by the chromosomal protein MBD1. *Mol Cell Biol* 20: 1394-1406.

NG, H.H., ZHANG, Y., HENDRICH, B., JOHNSON, C.A., TURNER, B.M., ERDJUMENT-BROMAGE, H., TEMPST, P., REINBERG, D. and BIRD, A. (1999). MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nat Genet* 23: 58-61.

NOMURA, T., KIMURA, M., HORII, T., MORITA, S., SOEJIMA, H., KUDO, S. and HATADA, I. (2008). MeCP2-dependent repression of an imprinted miR-184 released by depolarization. *Hum Mol Genet*. 17: 1192-1199.

OHKI, I., SHIMOTAKE, N., FUJITA, N., JEE, J., IKEGAMI, T., NAKAO, M. and SHIRAKAWA, M. (2001). Solution structure of the methyl-CpG binding domain of human MBD1 in complex with methylated DNA. *Cell* 105: 487-497.

OKANO, M., BELL, D.W., HABER, D.A. and LI, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99: 247-257.

OKANO, M., XIE, S. and LI, E. (1998a). Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat Genet* 19: 219-220.

OKANO, M., XIE, S. and LI, E. (1998b). Dnmt2 is not required for de novo and maintenance methylation of viral DNA in embryonic stem cells. *Nucleic Acids Res* 26: 2536-2540.

PAPAIT, R., PISTORE, C., NEGRI, D., PECORARO, D., CANTARINI, L. and BONAPACE, I.M. (2007). Np95 is implicated in pericentromeric heterochromatin replication and in major satellite silencing. *Mol Biol Cell* 18: 1098-1106.

PARK, J.I., KIM, S.W., LYONS, J.P., JI, H., NGUYEN, T.T., CHO, K., BARTON, M.C., DEROO, T., VLEMINCKX, K., MOON, R.T. *et al.* (2005). Kaiso/p120-catenin and TCF/beta-catenin complexes coordinately regulate canonical Wnt gene targets. *Dev Cell* 8: 843-854.

PEREZ-TORRADO, R., YAMADA, D. and DEFOSSEZ, P.A. (2006). Born to bind: the BTB protein-protein interaction domain. *Bioessays* 28: 1194-1202.

PROKHORTCHOUK, A., HENDRICH, B., JORGENSEN, H., RUZOV, A., WILM, M., GEORGIEV, G., BIRD, A. and PROKHORTCHOUK, E. (2001). The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. *Genes Dev* 15: 1613-1618.

PROKHORTCHOUK, A., SANSOM, O., SELFRIDGE, J., CABALLERO, I.M., SALOZHIN, S., AITHOZHINA, D., CERCHIETTI, L., MENG, F.G., AUGENLICHT, L.H., MARIADASON, J.M. *et al.* (2006). Kaiso-deficient mice show resistance to intestinal cancer. *Mol Cell Biol* 26: 199-208.

RUZOV, A., DUNICAN, D.S., PROKHORTCHOUK, A., PENNINGS, S., STANCHEVA, I., PROKHORTCHOUK, E. and MEEHAN, R.R. (2004). Kaiso is a genome-wide repressor of transcription that is essential for amphibian development. *Development* 131: 6185-6194.

RUZOV, A., SAVITSKAYA, E., HACKETT, J.A., REDDINGTON, J.P., PROKHORTCHOUK, A., MADEJ, M.J., CHEKANOV, N., LI, M., DUNICAN, D.S., PROKHORTCHOUK, E. *et al.* (2009). The non-methylated DNA-binding function of Kaiso is not required in early Xenopus laevis development. *Development* 136: 729-38.

SAITO, M. and 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.

SANSOM, O.J., BERGER, J., BISHOP, S.M., HENDRICH, B., BIRD, A. and CLARKE, A.R. (2003). Deficiency of Mbd2 suppresses intestinal tumorigenesis. *Nat Genet* 34: 145-147.

SARRAF, S.A. and STANCHEVA, I. (2004). Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. *Mol Cell* 15: 595-605.

SASAI, N., MATSUDA, E., SARASHINA, E., ISHIDA, Y. and KAWAICHI, M. (2005). Identification of a novel BTB-zinc finger transcriptional repressor, CIBZ, that interacts with CtBP corepressor. *Genes Cells* 10: 871-885.

SEKIMATA, M. and HOMMA, Y. (2004). Sequence-specific transcriptional repression by an MBD2-interacting zinc finger protein MIZF. *Nucleic Acids Res* 32: 590-597.

SHARIF, J., MUTO, M., TAKEBAYASHI, S., SUETAKE, I., IWAMATSU, A., ENDO,

T.A., SHINGA, J., MIZUTANI-KOSEKI, Y., TOYODA, T., OKAMURA, K. *et al.* (2007). The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450: 908-912.

SHEN, L., KONDO, Y., GUO, Y., ZHANG, J., ZHANG, L., AHMED, S., SHU, J., CHEN, X., WATERLAND, R.A. and ISSA, J.P. (2007). Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. *PLoS Genet* 3: 2023-2036.

SHI, Y., SAWADA, J., SUI, G., AFFAR EL, B., WHETSTINE, J.R., LAN, F., OGAWA, H., LUKE, M.P. and NAKATANI, Y. (2003). Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* 422: 735-738.

SPRING, C.M., KELLY, K.F., O'KELLY, I., GRAHAM, M., CRAWFORD, H.C. and DANIEL, J.M. (2005). The catenin p120ctn inhibits Kaiso-mediated transcriptional repression of the beta-catenin/TCF target gene matrilysin. *Exp Cell Res* 305: 253-265.

STANCHEVA, I., COLLINS, A.L., VAN DEN VEYVER, I.B., ZOGHBI, H. and MEEHAN, R.R. (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.

STANCHEVA, I., HENSEY, C. and MEEHAN, R.R. (2001). Loss of the maintenance methyltransferase, xDnmt1, induces apoptosis in *Xenopus* embryos. *EMBO J* 20: 1963-1973.

STANCHEVA, I. and MEEHAN, R.R. (2000). Transient depletion of xDnmt1 leads to premature gene activation in *Xenopus* embryos. *Genes Dev* 14: 313-327.

SUZUKI, M.M. and BIRD, A. (2008). DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* 9: 465-76.

TAKEBAYASHI, S., TAMURA, T., MATSUOKA, C. and OKANO, M. (2007). Major and essential role for the DNA methylation mark in mouse embryogenesis and stable association of DNMT1 with newly replicated regions. *Mol Cell Biol* 27: 8243-8258.

TAKIZAWA, T., NAKASHIMA, K., NAMIHIRA, M., OCHIAI, W., UEMURA, A., YANAGISAWA, M., FUJITA, N., NAKAO, M. and TAGA, T. (2001). DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev Cell* 1: 749-758.

TUDOR, M., AKBARIAN, S., CHEN, R.Z. and 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.

UNOKI, M., NISHIDATE, T. and NAKAMURA, Y. (2004). ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. *Oncogene* 23: 7601-7610.

VAN ROY, F.M. and MCCREA, P.D. (2005). A role for Kaiso-p120ctn complexes in cancer? *Nat Rev Cancer* 5: 956-964.

WALSH, C.P. and XU, G.L. (2006). Cytosine methylation and DNA repair. *Curr Top Microbiol Immunol* 301: 283-315.

WANG, Y., JORDA, M., JONES, P.L., MALESZKA, R., LING, X., ROBERTSON, H.M., MIZZEN, C.A., PEINADO, M.A. and ROBINSON, G.E. (2006). Functional CpG methylation system in a social insect. *Science* 314: 645-647.

WATANABE, S., ICHIMURA, T., FUJITA, N., TSURUZOE, S., OHKI, I., SHIRAKAWA, M., KAWASUJI, M. and NAKAO, M. (2003). Methylated DNA-binding domain 1 and methylpurine-DNA glycosylase link transcriptional repression and DNA repair in chromatin. *Proc Natl Acad Sci U S A* 100: 12859-12864.

WEBER, M., HELLMANN, I., STADLER, M.B., RAMOS, L., PAABO, S., REBHAN, M. and SCHUBELER, D. (2007). Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* 39: 457-466.

WEBER, A., MARQUARDT, J., ELZI, D., FORSTER, N., STARKE, S., GLAUM, A., YAMADA, D., DEFOSSEZ, P.A., DELROW, J., EISENMAN, R.N. *et al.* (2008). Zbtb4 represses transcription of P21CIP1 and controls the cellular response to p53 activation. *EMBO J* 27: 1563-74.

WOO, H.R., PONTES, O., PIKAARD, C.S. and RICHARDS, E.J. (2007). VIM1, a methylcytosine-binding protein required for centromeric heterochromatinization. *Genes Dev* 21: 267-277.

XU, G.L., BESTOR, T.H., BOURC'HIS, D., HSIEH, C.L., TOMMERUP, N., BUGGE, M., HULTEN, M., QU, X., RUSSO, J.J. and VIEGAS-PEQUIGNOT, E. (1999). Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* 402: 187-191.

YASUI, D.H., PEDDADA, S., BIEDA, M.C., VALLERO, R.O., HOGART, A., NAGARAJAN, R.P., THATCHER, K.N., FARNHAM, P.J. and LASALLE, J.M. (2007). Integrated epigenomic analyses of neuronal MeCP2 reveal a role for long-range interaction with active genes. *Proc Natl Acad Sci U S A* 104: 19416-19421.

YOON, H.G., CHAN, D.W., REYNOLDS, A.B., QIN, J. and WONG, J. (2003). N-CoR mediates DNA methylation-dependent repression through a methyl CpG binding protein Kaiso. *Mol Cell* 12: 723-734.

YOUNG, J.I., HONG, E.P., CASTLE, J.C., CRESPO-BARRETO, J., BOWMAN, A.B., ROSE, M.F., KANG, D., RICHMAN, R., JOHNSON, J.M., BERGET, S. *et al.* (2005). Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. *Proc Natl Acad Sci U S A* 102: 17551-17558.

ZEMACH, A. and GRAFI, G. (2007). Methyl-CpG-binding domain proteins in plants: interpreters of DNA methylation. *Trends Plant Sci* 12: 80-85.

ZHANG, Q., YOSHIMATSU, Y., HILDEBRAND, J., FRISCH, S.M. and GOODMAN, R.H. (2003). Homeodomain interacting protein kinase 2 promotes apoptosis by downregulating the transcriptional corepressor CtBP. *Cell* 115: 177-186.

ZHANG, Y., NG, H.H., ERDJUMENT-BROMAGE, H., TEMPST, P., BIRD, A. and REINBERG, D. (1999). Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev* 13: 1924-1935.

ZHAO, X., UEBA, T., CHRISTIE, B.R., BARKHO, B., MCCONNELL, M.J., NAKASHIMA, K., LEIN, E.S., EADIE, B.D., WILLHOITE, A.R., MUOTRI, A.R. *et al.* (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.