Methyl-CpG-binding Protein, MeCP2, Is a Target Molecule for Maintenance DNA Methyltransferase, Dnmt1*

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During mammalian cell division, DNA methylation patterns are transferred accurately to the newly synthesized DNA strand. This depends on maintenance DNA methyltransferase activity. DNA methylation can affect chromatin organization and gene expression by recruitment of histone deacetylases (HDACs). Here we show that the methyl-CpG binding protein, MeCP2, interacts directly with the maintenance DNA methyltransferase, Dnmt1. The region of MeCP2 that interacts with Dnmt1 corresponds to the transcription repressor domain which can also recruit HDACs via a corepressor, mSin3A. Dnmt1 can form complexes with HDACs as well as MeCP2. Surprisingly, the MeCP2-Dnmt1 complex does not contain the histone deacetylase, HDAC1. Thus, Dnmt1 takes the place of the mSin3A-HDAC1 complex, indicating that the MeCP2-interacting Dnmt1 does not bind to HDAC1. Further, we demonstrate that MeCP2 can form a complex with hemimethylated as well as fully methylated DNA. Immunoprecipitated MeCP2 complexes show DNA methyltransferase activity to hemimethylated DNA. These results suggest that Dnmt1 associates with MeCP2 in order to perform maintenance methylation in vivo. We propose that genome-wide and/or specific local DNA methylation may be maintained by the Dnmt1-MeCP2 complexes, bound to hemimethylated DNA. Dnmt1 may be recruited to targeted regions via multiple steps that may or may not involve histone deacetylases.

DNA methylation occurs at the fifth carbon position of cytosine in CpG dinucleotide sequences. In mammalian cells, 60–80% of CpG dinucleotides are methylated (1). These modifications are considered to be important for development (2, 3), genomic imprinting (4), and X chromosome inactivation through gene silencing (5, 6). Aberrant DNA methylation has been observed in cancer cells (7). The occurrence of DNA methylation is closely connected with chromatin organization and gene silencing via recruitment of HDACs (5). MeCP2, the first cloned Methyl-CpG binding protein, binds to single CpG dinucleotides that are symmetrically methylated (17), whereas other MBDs have little or no binding activity to single symmetrically methylated CpG dinucleotides (18). Thus, MeCP2 possesses higher affinity for all or most of the methylated CpG sites than other known MBDs. MeCP2 represses gene expression by recruiting mSin3A, which interacts with HDAC1 (19, 20). The tissue distribution of MeCP2 is ubiquitous, and its expression is relatively abundant (21, 22). Mutations of the MeCP2 gene cause loss of body weight and a neurological disorder in the mouse, which is consistent with MeCP2 mutations in Rett syndrome (23, 24). This indicates that MeCP2 is not essential for development but participates in epigenetic control of neuronal function.

Dnmt1 does not contain a methyl-CpG binding domain common to the MBD family (18), raising the question of how this enzyme is recruited to hemimethylated DNA and how it replicates the methylation pattern. We tested the hypothesis that the pattern of DNA methylation is replicated through recruitment of Dnmt1 by MeCP2, which binds to the methylated cytosine of the template DNA. Here we show that Dnmt1 interacts with MeCP2 directly, and this interaction is dependent upon the transcription repressor domain (TRD) of MeCP2. The Dnmt1-MeCP2 complex does not include HDAC1 but has maintenance methyltransferase activity. Moreover, MeCP2 can preferentially associate with both hemimethylated and fully methylated DNA under a physiological salt concentration condition. These results suggest that both Dnmt1 and
MeCP2 may contribute to maintenance of DNA methylation during DNA replication by multiple regulatory machineries including various protein-protein interactions.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—We subcloned rat Dnmt1 cDNA (25) into pcDNA2- MT (26) and then cloned Myc-tagged rat Dnmt1 cDNA and rat Dnmt1 cDNA into pcDNA3 to produce pcDNA3-Myc-Dnmt1. We cloned rat MeCP2 cDNA and internal deletion constructs (ΔMBD, ΔCRID, and ΔTRD) into pCMV2-FLAG (Sigma). Individual domains of rat MeCP2 were cloned into pGEX 4T-3 (Amersham Biosciences) by PCR using appropriate sets of primers. We cloned mouse HDAC1 cDNA into pHM (Roche Molecular Biochemicals). Mouse HDAC1 cDNA and internal deletion constructs (ΔMBD, ΔCRID, and ΔTRD) were then subjected to each DNA binding reaction for 1 h at 4 °C using 6 μg (30 μmol) of double-stranded oligonucleotides corresponding to E-cadherin CpG nucleotide sequences (28) (Sigma Genosys Japan KK). These were either nonmethylated, hemimethylated (on either the sense strand or on the antisense strand), or fully methylated, and all were used for immunoprecipitation at the 5'-ends. Sense strand has the sequence 5'-AGGCCGGCGACCACCCGCACCCGGGGC-3' (portion of methylated cytosines are underlined). Methylated oligonucleotides were generated at specific portions by using methylated precursors. Streptavidin-magnetic beads (Roche Molecular Biochemicals) were added to collect the biotinylated oligonucleotides with binding proteins, and incubation was continued with a rotary for 1 h at 4 °C. The beads were washed three times with Nonidet P-40 buffer (1 ml) and were subjected to Western blot analysis using anti-FLAG antibody (M2).

**Translation—**We transfected 293T cells with either Myc-Dnmt1 and FLAG-MeCP2 expression vectors, empty vector, and FLAG-MeCP2 expression vectors, empty vector alone, or FLAG-Dnmt1 expression vectors were lysed using Nonidet P-40 buffer as described above. After immunoprecipitation using anti-FLAG antibody, beads were washed three times with Nonidet P-40 buffer, and then the precipitates were assayed for methylation transferase activity in a 100-μl reaction solution containing a 32-bp hemimethylated oligonucleotide substrate (500 ng (25 pmol)), which is the same as described above (28), S-adenosyl-L-[methyl-3H]methionine (2 μl; 77 Ci/mmol; Amersham Biosciences), 50 mM Tris-Cl, pH 7.5, 5 mM EDTA, 50% glycerol, 5 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride. After incubation for 1 h at 37 °C or 4 °C, we removed unincorporated nuclides with Sephadex G-50 spin column (Amersham Biosciences) and determined incorporation of radioactivity in ASCII solvent (Amersham Biosciences) by liquid scintillation counting (13).

**RESULTS**

The majority of MeCP2 is concentrated on heterochromatin in the genome (29). Lysis under high salt concentration conditions results in extraction of all MeCP2 in the cell (17). However, a high salt extraction also disrupts weaker affinity interactions. The extraction conditions used here take advantage of excluding MeCP2 associated with tightly condensed chromatin such as heterochromatin and of selecting MeCP2 associated with relatively loose chromatin such as during DNA replication. In previous reports, an interaction of Dnmt1 with PCNA (11), DMAP1 (12), HDACs (19), and Rb (15) has been shown under the same physiological salt condition. To test an interaction of Dnmt1 with MeCP2, we co-transfected 293T cells with plasmid encoding Myc-tagged, full-length rat Dnmt1 (Myc-Dnmt1) and FLAG-tagged, full-length rat MeCP2 (FLAG-MeCP2). Then we lysed the cells using a lysis buffer with a typical physiological salt concentration and carried out immunoprecipitation with the anti-FLAG antibody, followed by Western blot analysis using the anti-Myc antibody. Dnmt1 and MeCP2 interacted in vivo (Fig. 1, A and B, lanes 4 and 8), and Dnmt1 was not immunoprecipitated in the absence of MeCP2 (Fig. 1, A and B, lanes 3 and 7). Endogenous MeCP2 was detectable under the same salt condition in mouse primary cells using an anti-MeCP2 antibody (data not shown). We then determined whether MeCP2 can bind directly to Dnmt1 in vitro by means of a GST pull-down assay using seven kinds of bacterially expressed and purified GST fusion MeCP2 proteins; GST 1–76, 77–161 (MBD), 162–206 (CRID), 207–310 (TRD), 311–403, 404–491, and wild type (residues 1–491) (Fig. 1C). Three separate domains of MeCP2, as well as full-length MeCP2 protein, were able to bind to in vitro translated full-length Dnmt1 (Fig. 1D, lanes 4–6 and 9). These associations were specific because Dnmt1 failed to bind to GST alone (Fig.
To confirm that the GST-fused individual domains of MeCP2 and full-length MeCP2 can bind to Dnmt1 prepared from mammalian cells, we transfected 293T cells with an empty vector or Myc-Dnmt1 expression vector. We lysed the cells and carried out GST pull-down assays using the lysate, followed by Western blot analysis using the anti-Myc antibody. Three domains (MBD, CRID, and TRD) of MeCP2 and full-length MeCP2 bound full-length Dnmt1 (Fig. 1E). These associations were specific because GST alone failed to bind to Dnmt1 (Fig. 1E, lane 4). Dnmt1 was not detectable from the lysate transfected with empty vector (Fig. 1E, odd-numbered lanes). These results indicate that Dnmt1 binds directly to MeCP2 in vitro (Fig. 1, D and E). Moreover, three different functional domains of MeCP2 could bind to Dnmt1 (Fig. 1, D [lanes 4–6] and E [lanes 8, 10, and 12]).

Dnmt1 possesses regulatory domains in the N-terminal region (10), which can form complexes with many proteins (e.g. DMAP1 (12), PCNA (11), Rb (14), and RFTS (30)). We next examined which domain of Dnmt1 binds to MeCP2 by GST pull-down assay using several Dnmt1 constructs with serial deletions from the C terminus. A bipartite nuclear localization sequence has previously been identified in the N-terminal region (NLS; residues 171–201, rat Dnmt1) (Fig. 2A) (30). All Dnmt1 constructs could be localized in the nucleus (Fig. 2A).

Lysates from 293T cells transfected with the mutant Dnmt1 constructs were provided to the assay. Most Dnmt1 fragments (residues 1–1,622, 1–1,414, 1–1,203, 1–1,014, 1–807, 1–608, 1–428, and 1–327) bound to GST-full-length MeCP2, and the three individual domains of MeCP2 (Fig. 2B). The binding activity of fragment 1–327 to MBD was weaker than others. In contrast, Dnmt1 constructs 1–263 and 1–201 bound incompletely to the MeCP2 protein (Fig. 2B). These results suggest that residues 264–326 of Dnmt1 are necessary for the interaction with MeCP2.

The MBD, CRID, and TRD domains of MeCP2 can bind independently to Dnmt1 (Fig. 2B). To examine which domain...
plays a central role for an interaction with Dnmt1 in vivo, we generated a series of FLAG-tagged internal deletion mutants of MeCP2 (FLAG-Mecp2, ΔMBD, ΔCRID, and ΔTRD), all of which localize in the nucleus (Fig. 3A). We co-transfected 293T cells with Myc-Dnmt1 and with empty vector, FLAG-Mecp2, FLAG-ΔMBD, FLAG-ΔCRID, or FLAG-ΔTRD. We lysed the cells and carried out immunoprecipitation with the anti-FLAG antibody, followed by Western blot analysis using the anti-Myc antibody. Full-length Dnmt1 and MeCP2 interacted as in Fig. 1A (Fig. 3B, lanes 1 and 2, column 1). Dnmt1 interacted specifically with ΔMBD and ΔCRID (Fig. 3B, lanes 3 and 4), but the interaction of Dnmt1 with ΔTRD decreased strikingly (Fig. 3B, lane 5, column 4). These results indicate that the TRD plays a central role for an interaction with Dnmt1 in vivo. Deletion of MBD reduced the binding activity to Dnmt1 (Fig. 3B, lane 3, column 2). This may suggest that MBD also

**Fig. 2. Identification of the domain in Dnmt1 that interacts with MeCP2.** A, maps of the Dnmt1 deletion constructs. Amino acid positions are indicated on the left. A summary of nuclear localization of the constructs and their binding activities with GST-MeCP2 is shown on the right. MBD, MeCP2 interaction domain (30). B, Western blot analysis of various Myc-Dnmt1 proteins pulled down from lysate of 293T cells transfected with the Myc-Dnmt1 deletion constructs (based on pcDNA3-Myc-Dnmt1) as shown in 293T cells transfected with the Myc-Dnmt1 deletion constructs. A summary of binding activities with the full-length Dnmt1 are shown to the right. B, Western blot analysis showing co-immunoprecipitation of full-length Myc-Dnmt1 with FLAG-MeCP2 internal deletion mutants. 293T cells were co-transfected with 10.5 μg of pcDNA3-Myc-Dnmt1 and 10.5 μg of empty vector, pCMV2-FLAG-MeCP2, or the pCMV2-FLAG-MeCP2 mutants as indicated. A, Top panel, immunoprecipitates with anti-FLAG antibody (M2); the presence of Myc-Dnmt1 was visualized by Western blot analysis using anti-Myc antibody (9E10). Middle and bottom panels, immunoprecipitates with anti-FLAG antibody and whole cell extracts (WCE) were subjected to Western blot analysis using anti-FLAG antibody and anti-Myc antibody to confirm the expression. The right graph shows relative Dnmt1 binding activity of wild type and mutant MeCP2. Numbers shown are Dnmt1 binding activity relative to immunoprecipitated FLAG-MeCP2 or mutants. Dnmt1 binding activity of wild type MeCP2 is represented as 1. Intensity of protein signal is quantitated by using densitometry ChemiImager 4800 software (Alpha Innotech Corp.). Similar results were obtained from two independent experiments. C, Western blot analysis showing co-immunoprecipitation of full-length HDAC1 with Dnmt1, MeCP2, and Rb. 293T cells were co-transfected with 10.5 μg of pHM-HA-HDAC1 and either 10.5 μg of pcDNA3-Myc-Dnmt1, pCMV2-FLAG-MeCP2, pME18S SR-Rb, or the corresponding empty vectors. Cell extracts were then precipitated with anti-Myc, anti-FLAG, or anti-Rb antibodies as appropriate. Top panel, immunoprecipitate (IP) with various antibodies; bottom panel, whole cell extracts. D, Western blot analysis showing that the complex MeCP2-Dnmt1 does not contain HDAC1. 293T cells were co-transfected with 10 μg of pCMV2-FLAG-MeCP2, 10 μg of pcDNA3-Myc-Dnmt1, and 10 μg of pHM-MA-HDAC1 in culture dishes (100-mm diameter) as indicated. Cell extracts were then precipitated with anti-FLAG antibody. Whole cell extracts and the immunoprecipitates were subjected to Western blot analysis using anti-Myc, anti-HA, and anti-FLAG antibodies.
contributes for an interaction of MeCP2 and Dnmt1. A previous study has reported that Dnmt1 associates with HDAC1 (13). MeCP2 also interacts with HDAC1 by forming a corepressor complex including mSin3A (19, 20). In our system, Dnmt1, MeCP2, and Rb all interacted with HDAC1 (Fig. 3C). To examine whether a Dnmt1-MeCP2 complex includes HDAC1, we co-transfected 293T cells with plasmids containing FLAG-MeCP2, Myc-Dnmt1, and HA-HDAC1. Immunoprecipitates using anti-FLAG antibody from lysates including FLAG-MeCP2, Myc-Dnmt1, and HA-HDAC1 did not include HA-HDAC1 (Fig. 3D, lane 7), whereas immunoprecipitates from lysates with FLAG-MeCP2 and HA-HDAC1 included HA-HDAC1 (Fig. 3D, lane 6). These results suggest that an interaction of Dnmt1 with MeCP2 is stronger than that of mSin3A with MeCP2, and the Dnmt1-MeCP2 complex excludes HDAC1 from both Dnmt1-HDAC1 and MeCP2-mSin3A-HDAC1 components.

If the interaction of Dnmt1 with MeCP2 is involved in replicating patterns of methylation, MeCP2 would be predicted to bind to hemimethylated DNA as well as to fully methylated DNA. We used synthetic biotinylated oligonucleotides corresponding to the in vivo CpG island of the E-cadherin gene (28) to investigate this. We confirmed that hemimethylated double-stranded oligonucleotides do not form a hairpin loop structure in the methylated strand using DNA electrophoresis under nondenaturing conditions and by oligonucleotide secondary structure predictions using Vector NTI software (data not shown). By means of an in vitro DNA pull-down assay (Fig. 4A), we found that MeCP2 bound not only to fully methylated oligonucleotides but also to hemimethylated ones (Fig. 4B). These associations were specific because MeCP2 failed to bind to the nonmethylated oligonucleotides of the same sequence (Fig. 4B) (17).

This interaction led us to consider that the Dnmt1-MeCP2 complex could be associated with maintenance DNA methyltransferase activity. To test this idea, we performed transfec tions with either FLAG-MeCP2 and empty vector or FLAG-MeCP2 and Myc-Dnmt1 expression vectors. We lysed the cells, immunoprecipitated FLAG-MeCP2 with anti-FLAG antibody from the lysates, and then assayed the immunoprecipitates for maintenance methyltransferase activity. Immune complexes from cells transfected with FLAG-MeCP2 and Myc-Dnmt1 expression vectors possessed higher methyltransferase activity than cells transfected with FLAG-MeCP2 expression and empty vectors (Fig. 4C, columns 1 and 2). Immunoprecipitates of Dnmt1 were also assayed as a positive control and indicated sufficient enzymatic activity in this system (Fig. 4C, columns 3 and 4). Methyltransferase activity of Dnmt1 to hemimethylated DNA is ∼50-fold higher than de novo methyltransferase activity (9). Thus, these results indicate that MeCP2-interacting Dnmt1 has significant maintenance DNA methyltransferase activity and that MeCP2 does not vanish Dnmt1 enzymatic activity. When this enzymatic reaction was also tested at 4°C, maintenance DNA methyltransferase activity was equal to that of cells transfected with an empty vector (data not shown). DNA pull-down assay and methyltransferase assay were performed using the same hemimethylated double-stranded oligonucleotides (see “Experimental Procedures”). These results indicate that hemimethylated double-stranded oligonucleotides do not become fully methylated by endogenous MeCP2 under the DNA pull-down condition.

DISCUSSION

Our data indicate a direct interaction between Dnmt1 and MeCP2. This interaction prevents the association of HDAC1 with MeCP2. MeCP2 associates with hemimethylated as well as fully methylated DNA in vitro. The MeCP2-Dnmt1 complex possesses maintenance DNA methyltransferase activity that is

**FIG. 4.** MeCP2 recognizes both fully methylated and hemimethylated oligonucleotides, and the complex MeCP2-Dnmt1 has maintenance methyltransferase activity. A, schematic diagrams of synthetic biotinylated, modified, double-stranded oligonucleotides and of the DNA pull-down assay, which uses streptavidin-conjugated beads to trap the biotinylated oligonucleotides and any proteins bound to them. B, Western blot analysis for MeCP2 from the DNA pull-down assay. 293T cells were transfected with empty vector (V) or pCMV2-FLAG-MeCP2 (M) and lysed. Synthetic double-stranded oligonucleotides were added to the lysate, incubated, and retrieved with streptavidin-conjugated beads. N, nonmethylated, double-stranded oligonucleotides; Hemi Sense, sense strand methylated; Hemi Anti, antisense strand methylated; Full, both sense and antisense strands methylated. C, maintenance methyltransferase activity associated with MeCP2. 293T cells were co-transfected with various combinations of 10.5 μg of pCMV2-FLAG-MeCP2 and 10.5 μg of pcDNA3-Myc-Dnmt1 or 21 μg of pCMV2-FLAG-Dnmt1 as indicated. Cells were lysed, and whole cell extracts were then precipitated with anti-FLAG antibody. Immunoprecipitates were washed and assayed for methyltransferase activity by using S-adenosyl-L-[methyl-3H]methionine at 37°C. Activity is read as cpm of 3H incorporated into a hemimethylated double-stranded oligonucleotide substrate (28). Values are expressed as -fold above immunoprecipitated complexes using anti-FLAG antibody from vector alone-transfected 293T cells (Vector column).
dependent upon Dnmt1, demonstrating that the Dnmt1-MeCP2 complex could be involved in maintenance of DNA methylation. Histone deacetylase activity associates with Rb (31), Dnmt1 (13), and MBD proteins such as MeCP2 (19), MBD1 (32), and MBD2 (33). Thus, Rb, Dnmt1, and MBDs complexes have histone deacetylase activity. Our findings suggest that histone deacetylation at least partly mediates various nucleosomal events such as chromatin integrity and gene regulation. We propose that histone deacetylase is not essential for maintenance DNA methyltransferase activity in the Dnmt1-MeCP2 complex.

An N-terminal domain of Dnmt1 interacts with cell cycle-related molecules such as PCNA (11) and Rb (14, 15). The RFTS domain of Dnmt1 is near the RFTS, and deletion of the RFTS (amino acids 328–378) reduces, but does not eliminate, interaction with MeCP2 (Fig. 2). These results suggest that interaction of the two may be supported by the RFTS. Subcellular localization of Dnmt1 may be regulated by interplay of the MeCP2 interaction domain and RFTS.

The connection between DNA methylation and histone acetylation will mainly affect chromatin organization by maintaining a stable epigenetic state and remodeling chromatin, but the enzymatic activity of DNA methyltransferase itself may be regulated by molecules such as MeCP2 that bind to DNA. Some studies suggest that the Dnmt1 enzymatic reaction may take place at classical replication foci (30), but little is known about the molecular mechanism of how CpG methylation is maintained. The discovery that Dnmt1 directly interacts with MeCP2 will help us to understand this issue. In proliferating cells, expression of Dnmt1 is induced as DNA synthesis approaches (34). Recent studies and our data imply a mechanism by which DNA methylation is maintained as described below (Fig. 5). First, Dnmt1 is recruited to replication foci by DMAP1 and/or PCNA (11, 12). When replication forks with the PCNA clamp structure progress (35), MeCP2 bound to symmetrically methylated DNA is released. Free MeCP2 reassociates with hemimethylated, newly synthesized DNA. Dnmt1 interacts with MeCP2, recognizing the complementary nonmethylated CpG dinucleotides, and transfers a methyl group by the enzymatic reaction. Then MeCP2 may change its conformation promptly. Finally, MeCP2 would bind to fully methylated CpG dinucleotides to maintain gene silencing and chromatin structures by recruitment of HDACs (33). In our model of maintenance of DNA methylation, these properties of DNA binding activity and interaction with some molecules may be regulated by post-translational modifications such as phosphorylation during DNA replication. In this regard, we found that both Dnmt1 and MeCP2 are phosphorylated at multiple sites in vivo.2 Thus, Dnmt1 might require MeCP2 to carry out genome-wide and/or local maintenance DNA methylation.

Although mutations of DNA methyltransferase and methyl-CpG-binding protein family genes affect loss of DNA methylation in development (36) and/or cellular function (23, 24), little is understood regarding which protein complexes are involved in those processes. Our discovery implicates that Dnmt1 and MeCP2 may interplay in related pathways in both DNA methylation and histone modification. An interaction of Dnmt1 and MeCP2 is dependent upon the TRD in the C-terminal region of MeCP2 in vivo. Therefore, the MBD of MeCP2 within the complex could bind hemimethylated DNA, targeting Dnmt1 activity to MeCP2-responsive genes. One important question that remains to be answered about the Dnmt1-MeCP2 interaction is whether there is a specific target for the genome or other molecules to be involved in DNA methylation and chromatin remodeling. Future studies of the functions of complexes formed by DNA methyltransferases and methyl-CpG binding

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Fig. 5. Schematic diagram of the molecular mechanism of methylation inheritance and transcriptional repression. The complex, MeCP2-mSin3A-HDAC1/2, bound to fully methylated, double-stranded DNA, represses gene activation and organizes chromatin integrity. During DNA replication, the Dnmt1-MeCP2 complexes bind to hemimethylated CpG dinucleotides in double-stranded DNA and methylate the cytosines in the newly synthesized strands. MeCP2 recognizes fully methylated, double-stranded DNA, and the complex MeCP2-mSin3A-HDAC1/2 is formed.
domain proteins will be required to determine which complexes are responsible for tissue-specific organization and remodeling of chromatin structure and gene regulation by DNA methylation and histone modification in normal development and epigenetic diseases.

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REFERENCES
1. Razin, A., and Szyf, M. (1984) Biochim. Biophys. Acta 782, 331–342
2. Li, E., Bestor, T. H., and Jaenisch, R. (1992) Cell 69, 915–926
3. Shiota, K., and Yanagimachi, R. (2002) Differentiation 69, 162–166
4. Tucker, K. L., Beard, C., Dausmann, J., Jackson-Grusby, L., Laird, P. W., Lei, H., Li, E., and Jaenisch, R. (1996) Genes Dev. 10, 1008–1020
5. Bird, A. (2002) Genes Dev. 16, 6–21
6. Li, E. (2002) Nat. Rev. Genet. 3, 662–673
7. Baylin, S. B., and Herman, J. G. (2000) Trends Genet. 16, 168–174
8. Burgers, W. A., Fuku, P., and Kouzarides, T. (2002) Trends Genet. 18, 275–277
9. Okano, M., Xie, S., and Li, E. (1998) Nat. Genet. 19, 219–222
10. Bestor, T. H. (2000) Hum. Mol. Genet. 9, 2395–2402
11. Chuang, L. S., Ian, H. I., Koh, T. W., Ng, H. H., Xu, G., and Li, B. F. (1997) Nat. Genet. 16, 331
12. Rountree, M. R., Bachman, K. E., and Baylin, S. B. (2000) Nat. Genet. 25, 269–277
13. Fuku, P., Burgers, W. A., Brehm, A., Hughes-Davies, L., and Kouzarides, T. (2000) Nat. Genet. 24, 88–91
14. Robertson, K. D., Att-St-Ali, S., Yokochi, T., Wade, P. A., Jones, P. L., and Wolffe, A. P. (2000) Nat. Genet. 25, 338–342
15. Pradhan, S., and Kim, G. D. (2002) EMBO J. 21, 779–788
16. Wade, P. A. (2001) Bioessays 23, 1131–1137
17. Lewis, J. D., Meehan, R. R., Herzel, W. J., Maurer-Fogy, I., Jeppesen, P., Klein, F., and Bird, A. (1992) Cell 69, 805–914
18. Hendrich, B., and Bird, A. (1998) Mol. Cell. Biol. 18, 6538–6547
19. Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N., and Bird, A. (1998) Nature 393, 386–389
20. Jones, P. L., Veenastra, G. J., Wade, P. A., Vermaak, D., Kass, S. U., Landsberger, N., Strouboulis, J., and Wolffe, A. P. (1998) Nat. Genet. 18, 187–191
21. D’Esposito, M., Quaderi, N. A., Ciccodicola, A., Bruni, P., Esposito, T., D’Urso, M., and Brown, S. D. (1996) Mamm. Genome 7, 533–535
22. Cott, J. F., Sedlacek, Z., Bachner, D., Delius, H., and Pouet, A. (1999) Hum. Mol. Genet. 8, 1253–1262
23. Guy, J., Hendrich, B., Holmes, M., Martin, J. E., and Bird, A. (2001) Nat. Genet. 27, 322–326
24. Chen, R. Z., Akbarian, S., Tudor, M., and Jaenisch, R. (2001) Nat. Genet. 27, 327–331
25. Kimura, H., Takada, T., Tanaka, S., Ogawa, T., and Shiota, R. (1998) Biochem. Biophys. Res. Commun. 253, 495–501
26. Endo, T. A., Masuhr, M., Yokouchi, M., Suzuki, R., Sakamoto, H., Mitsu, K., Matsumoto, A., Tanimura, S., Ohtsubo, M., Misawa, H., Miyazaki, T., Leonor, N., Taniguchi, T., Fujita, T., Kanakura, Y., Komiy, S., and Yoshimura, A. (1997) Nature 387, 921–924
27. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
28. Rhee, I., Jair, K. W., Yen, E. W., Lengauer, C., Herman, J. G., Kinzler, K. W., Vogelstein, B., Baylin, S. B., and Schuebel, K. E. (2000) Nature 404, 1003–1007
29. Nan, X., Tate, P., Li, E., and Bird, A. (1996) Mol. Cell. Biol. 16, 414–421
30. Leonhardt, H., Page, A. W., Weier, H. U., and Bestor, T. H. (1992) Cell 71, 865–873
31. Brehm, A., Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J., and Kouzarides, T. (1998) Nature 391, 697–699
32. Ng, H. H., Jeppesen, P., and Bird, A. (2000) Mol. Cell. Biol. 20, 1384–1406
33. Bird, A. P., and Wolffe, A. P. (1999) Cell 99, 451–454
34. Szyf, M., Bozovic, V., and Tanigawa, G. (1991) J. Biol. Chem. 266, 10027–10030
35. Yong, X. P., O’Rourke, R., D’Onn, M., and Kuriyama, J. (1992) Cell 69, 425–437
36. Lei, H., Oh, S. P., Otsou, M., Juttermann, R., Goss, K. A., Jaenisch, R., and Li, E. (1996) Development 122, 3139–3205