Identification and Characterization of the DNA Binding Domain of CpG-binding Protein*

Received for publication, July 27, 2001, and in revised form, September 17, 2001

Published, JBC Papers in Press, September 25, 2001, DOI 10.1074/jbc.M107179200

Jeong-Heon Lee‡, Kui Shin Voo§§, and David G. Skalnik¶

From the Herman B Wells Center for Pediatric Research, Section of Pediatric Hematology/Oncology and the Departments of Pediatrics and Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202

CpG-binding protein is a transcriptional activator that exhibits a unique DNA binding specificity for unmethylated CpG motifs. CpG-binding protein contains a cysteine-rich CXXC domain that is conserved in DNA methyltransferase 1, methyl binding domain protein 1, and human trithorax. In vitro DNA binding assays reveal that CpG-binding protein contains a single DNA binding domain comprised of the CXXC domain and a short carboxyl extension. Specific mutation to alanine of individual conserved cysteine residues within the CXXC domain abolishes DNA binding activity. Denaturation/renaturation experiments in the presence of various metal cations demonstrate that the CXXC domain requires zinc for efficient DNA binding activity. Ligand selection of high affinity binding sites from a pool of degenerate oligonucleotides reveals that CpG-binding protein interacts with a variety of sequences that contains the CpG dinucleotide with a consensus binding site of (A/C)CpG(A/C). Mutation of the CpG motif(s) present within ligand-selected oligonucleotides ablates the interaction with CpG-binding protein, and mutation to thymine of the nucleotides flanking the CpG motifs reduces the affinity of CpG-binding protein. Hence, a CpG motif is necessary and sufficient to comprise a binding site for CpG-binding protein, although the immediate flanking sequence affects binding affinity.

The CpG dinucleotide represents an important regulatory component of mammalian genomes. The cytosine of this dinucleotide serves as the target for methylation via the action of DNA methyltransferases. Methylated DNA is correlated with transcriptionally inactive genes, whereas actively expressed genes are generally hypomethylated (1). It has also been suggested that cytosine methylation represents a defense mechanism to silence parasitic repetitive DNA elements present in mammalian genomes (2). Methylation patterns inherited from gametes are generally erased during early embryogenesis (morula) followed by a wave of de novo DNA methylation in the blastocyst upon implantation (3). The CpG dinucleotide is underrepresented in mammalian genomes (5–10% of expected frequency), presumably because of the propensity of 5-methylcytosine to undergo spontaneous deamination to form thymine. Approximately 50% of human genes are associated with CpG islands (1), which contain the statistically expected frequency of CpG dinucleotides. This may reflect the fact that CpG motifs near widely expressed genes are generally hypomethylated.

Cytosine methylation also plays an important role in the process of genomic imprinting, in which paternal and maternal alleles of a gene exhibit distinct patterns of methylation and expression (4), and X-chromosome inactivation, in which one X-chromosome in each cell of a female becomes transcriptionally inactivated during early development (5). Appropriate cytosine methylation is essential for normal mammalian development. Individual ablation of the DNA methyltransferase genes DNMT1,1 DNMT3a, or DNMT3b leads to a disruption of somatic methylation (6, 7). Furthermore, mutations in Dnmt3b that are predicted to partially inhibit function are associated with the ICF (immunodeficiency, centromere instability, and facial anomalies) syndrome in humans (8). Also, mutations in the methyl-CpG-binding protein MeCP2 lead to Rett’s syndrome, a progressive neurodegenerative disorder (9). Finally, hypermethylation of tumor suppressor genes is commonly observed in human cancer (10).

A number of DNA binding factors have been reported that bind to methylated CpG motifs and function as transcriptional repressors (11). These include MeCP2, methyl binding domain (MBD) protein 1, MBD2, and MBD4. Each of these factors contains a conserved methyl-CpG binding domain (MBD) but otherwise exhibits little sequence similarity. A fifth protein, MBD3, also contains the MBD domain but has not been shown to bind to methylated DNA. MBD2 and MBD3 are components of the histone deacetylase complexes MeCP1 and Mi-2, respectively (12, 13), thus linking cytosine methylation with histone acetylation and providing a unifying framework for the control of chromatin structure and gene regulation.

A major unanswered question is how specific CpG motifs become methylated during development and how CpG islands remain hypomethylated despite an open chromatin configuration and free access to DNA methyltransferases. Others have noted that the ubiquitous transcription factor Sp1 binds to sites within some CpG islands (its consensus recognition site is GGGGCGG) and protects the CpG island of the adenine phosphoribosyltransferase gene from cytosine methylation (14, 15). This has led to a model in which DNA binding proteins such as Sp1 may protect CpG islands from methylation. How-

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

* This work was supported by National Institutes of Health Grant CA58947 (to D. G. S.) and by the Riley Memorial Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Contributed equally to this work.

§ Supported by a post-doctoral fellowship awarded by the Arthritis Foundation.

¶ To whom correspondence should be addressed: Wells Center for Pediatric Research, Cancer Research Bldg., 1044 W. Walnut St., Rm. 472, Indianapolis, IN 46202. Tel.: 317-274-8977; Fax: 317-274-8928; E-mail: dskalnik@iupui.edu.

The abbreviations used are: DNMT, DNA methyltransferase; CGBP, CpG-binding protein; EMSA, electrophoretic mobility shift assay; HRX, human trithorax; MBD, methyl binding domain; PHD, plant homeodomain; PCR, polymerase chain reaction.

1 The abbreviations used are: DNMT, DNA methyltransferase; CGBP, CpG-binding protein; EMSA, electrophoretic mobility shift assay; HRX, human trithorax; MBD, methyl binding domain; PHD, plant homeodomain; PCR, polymerase chain reaction.

This paper is available on line at http://www.jbc.org

Vol. 276, No. 48, Issue of November 30, pp. 44669–44676, 2001
Printed in U.S.A.
ever, Sp1 is not required for maintaining hypomethylation of CpG islands because CpG islands remain hypomethylated after disruption of the Sp1 gene in mice (16).

We have previously described a novel transcriptional activating DNA binding protein termed CGBP-binding protein (CGBP) that specifically binds to sequences that contain unmethylated CpG dinucleotides (17). Hence, CGBP may play a role both in the expression of genes associated with CpG islands as well as in the regulation of cytosine methylation. Inspection of the CGBP sequence reveals the presence of several domains, including two copies of the plant homeodomain (PHD), a coiled-coil domain, basic and acidic stretches, and a highly conserved cysteine-rich CXXC domain that is also found in human trithorax (HRX, also known as MLL or ALL-1), MLL-2, DNMT1, and MBD1 (17–19). In these studies we determine that the CXXC domain comprises the sole DNA binding domain of CGBP and identify the consensus DNA binding sequence for CGBP.

**EXPERIMENTAL PROCEDURES**

**Materials.—**Oligonucleotides were purchased from Life Technologies Inc. by EMSA obtained from Promega Corporation. Bio-Rad and deoxyribonucleotides and poly(dA-dT) were obtained from Amersham Pharmacia Biotech. The ECL Western blotting detection system was purchased from Amersham Life Science. Nicotinamide was obtained from Micron Separations Inc. (Westborough, MA). The Bradford protein assay reagent was obtained from Bio-Rad. The pQE9 6His-tag expression vector was obtained from Qiagen Inc. (Valencia, CA). All other reagents were obtained from Sigma or Fisher.

**Oligonucleotides Used for in Vitro CGBP Binding Assays.—**The following oligonucleotides were synthesized as complementary sequences, annealed, and tested as CGBP binding sites in electrophoretic mobility shift assays (EMSA). Only the upper strand of each sequence is shown, and the CpG motifs and flanking sequences are underlined: CGBP, CCGGCTGGACCGAGATTTAACCAAAACGAGCAC; CGBP-17, CAGAGGCTGACCCAAGGAAACATCGA-GAGACACTGGGAATTC; CGBP-11, CAGAGGCTGACCCAAGGAAACATCG-A-GAGACACTGGGAATTC; CGBP-32, TCTGGTGGCGATAACTAAAGAAACATCGAGAGACACTGGGAATTC; CGBP-48-Mut2, TCTGGTGGCGATAACTAAAGAAACATCGAGAGACACTGGGAATTC; CGBP-48-Mut3, TCTGGTGGCGATAACTAAAGAAACATCGAGAGACACTGGGAATTC. The CGBP binding site oligonucleotides were made by utilising deoxyribonucleotides and poly(dA-dT) enzymes and primer extension. Recovered DNA fragments were subcloned into plasmids and sequenced to confirm the orientation and the start codon of each fragment. The CGBP binding site oligonucleotides were made using sequencing primers and fused to an amino acid sequence of the selected fragments are underlined. The oligonucleotides were made double-stranded by hybridization with end-labeled primer complementary to the downstream anchor sequence and elongation for 30 min at 37 °C with deoxyribonucleotide triphosphates and Klenow DNA polym-
Analysis of the DNA Binding Domain of CpG-binding Protein  

Identification of the CGBP DNA Binding Domain—Experiments were performed to identify the domain(s) of CGBP responsible for the unique DNA binding specificity for sequences containing unmethylated CpG dinucleotides. Overlapping fragments of human CGBP were expressed as 6×His-tagged proteins and tested for DNA binding activity in EMSA using the previously described CpG-pos binding site of CGBP as a probe (17). The CGBP DNA binding domain was truncated to amino acids 106–285, whereas protein fragments containing amino acids 1–153 or 231–656 fail to bind the DNA probe (Fig. 1B, lanes 4 and 5). Each of the 6×His-tagged CGBP protein fragments was successfully expressed and recovered from E. coli, as demonstrated by Western blot analysis of each protein sample (Fig. 1C). Multiple bands in some samples presumably reflect partial proteolysis. Hence, the DNA binding domain of CGBP resides within amino acids 106–285. Furthermore, the absence of DNA binding activity by overlapping CGBP fragments suggests that CGBP contains a single DNA binding domain that resides within amino acids 153–231, a fragment that contains the CXXC domain.

Additional studies were conducted on smaller fragments of CGBP to more precisely define the DNA binding domain (Fig. 2A). As predicted from the results presented in Fig. 1, the 142–250-amino acid region of CGBP exhibits DNA binding activity (Fig. 2B, lane 2). The 162–250-amino acid fragment of CGBP, which is truncated to within 2 residues of the amino end of the CXXC domain, continues to exhibit DNA binding activity (Fig. 2B, lane 3). Successive truncations of the carboxyl terminus reveals that a fragment as small as amino acids 162–221 exhibits DNA binding activity (Fig. 2B, lanes 4 and 5). This 60-amino acid fragment contains the CXXC domain as well as 15 residues of the carboxyl flanking region. Further truncation of 9 residues from the carboxyl tail (leaving amino acids 162–212) ablates DNA binding activity (Fig. 2B, lane 6). Each 6×His-tagged protein was successfully recovered from E. coli, as determined by Western blot analysis presented below the EMSA result (Fig. 2B). Hence, a 60-amino acid fragment of CGBP (amino acids 162–221) that contains the CXXC domain comprises the DNA binding domain.
Native CGBP binds specifically to DNA containing unmethylated CpG motifs (17). Additional studies were performed to determine whether the minimal DNA binding domain of CGBP retains this DNA binding specificity. Fig. 2 demonstrates that the EMSA complex produced by the 162–221 amino acid fragment of CGBP is efficiently disrupted by competition with oligonucleotide homologous to the probe (Fig. 2C, lanes 3–5) but not by a similar sequence (CpG-neg) that lacks the CpG motif (Fig. 2C, lanes 6–8). Importantly, the complex is also not disrupted by the CpG-pos oligonucleotide after methylation in vitro (Fig. 2C, lanes 9–11). Hence, the 162–221-amino acid fragment of CGBP exhibits a DNA binding specificity indistinguishable from that previously described for CGBP (17).

Site-specific mutagenesis was performed to determine if an intact CX2C domain is required for the DNA binding activity exhibited by the 142–250-amino acid fragment of CGBP. Fig. 3A presents a sequence alignment of eight reported CX2C domains (17–19). Eight of the 10 perfectly conserved residues are cysteines. We chose to mutate to alanine either the first or last cysteine residue. Each mutated form of CGBP was purified from E. coli as a 6xHis-tagged protein, and DNA binding activity to the CpG-pos probe was analyzed by EMSA (Fig. 3B). Mutation to alanine of either cysteine 169 or cysteine 208 completely abolished the DNA binding activity of this fragment of CGBP (Fig. 3B, lanes 2 and 3). Successful recovery of each 6xHis-tagged protein was confirmed by Western blot analysis,
as shown below the EMSA result. We conclude that an intact CXCC domain is required for the DNA binding activity of CGBP.

The structure of the CXCC domain is distantly related to a zinc finger, although it does not exactly fit the consensus arrangement of cysteines and histidines. Furthermore, the CXCC domain from DNMT1 has been demonstrated to bind zinc (25). We examined whether zinc is required for the observed DNA binding activity of the CXCC domain of CGBP. Purified 6×His-tagged CGBP fragment containing amino acids 162–221 was denatured in the presence of EDTA and EGTA and then renatured in the presence of various divalent metal cations. Fig. 4 demonstrates that the addition of zinc to the renaturation buffer (lane 7) is required to reconstitute efficient CGBP DNA binding activity to the CpG-pos probe.

**Identification of the Consensus DNA Binding Site of CGBP**—Previous work demonstrated that CGBP binds to DNA elements containing an unmethylated CpG dinucleotide (17). Experiments were performed to determine the consensus DNA binding site of CGBP. The 6×His-tagged CGBP protein fragment containing amino acids 162–221 was denatured in the presence of EDTA and EGTA and then renatured in the presence of various divalent metal cations. Fig. 4 demonstrates that the addition of zinc to the renaturation buffer (lane 7) is required to reconstitute efficient CGBP DNA binding activity to the CpG-pos probe.
No consensus sequence was otherwise apparent from this alignment, although adenine nucleotides are over-represented, composing 46% of the total nucleotides present in the degenerate region of the oligonucleotides.

All but one of the recovered sequences (oligonucleotide 38) contain additional CpG motifs elsewhere within the degenerate region of the oligonucleotide. Thirty-five of the recovered oligonucleotides carry a single additional CpG motif within the body of the degenerate sequence. Alignment of these sequences at the internal CpG reveals a similar preference for adenine or cytosine nucleotides at the positions immediately adjacent to the CpG motif and a general preference for adenine-rich DNA elements (Fig. 5B). Hence, the consensus binding site for CGBP appears to be (A/C)CpG(A/C).

Additional experiments were performed to directly assess whether ligand selection resulted in the recovery of high affinity binding sites for CGBP from the pool of degenerate oligonucleotides. Sequence 48 (Fig. 5A) contains two CpG motifs, both of which are present within the context of the consensus flanking sequence CCpGA. This DNA sequence disrupts the CGBP/CpG-pos probe EMSA complex more efficiently than does the homologous CpG-pos competitor, which carries a single CpG motif in the non-consensus context of CCpGG (Fig. 6, compare lanes 3–5 to lanes 6–8). As expected, the CpG-pos oligonucleotide competitor fails to disrupt the CGBP complex (Fig. 6, lanes 9–11). These results indicate that the ligand-selection process recovered high affinity CGBP binding sites.

Studies were performed to test the hypothesis that CGBP requires CpG dinucleotides for binding to the ligand-selected oligonucleotides and that adjacent adenine or cytosine nucleotides contributes to optimal binding affinity. Three ligand-selected sequences were chosen for analysis (Fig. 7A); they are sequence 48, which was described above and contains two CpG motifs; sequence 38, which contains a single CpG motif in the context of flanking cytosine and adenine nucleotides (CCpGA); and sequence 32, which contains two CpG motifs in the context of non-consensus flanking sequences (GCpGA and TCpGA).
DISCUSSION

We previously utilized a ligand-screening approach to clone CGBP, a novel transcriptional activator that binds specifically to DNA elements containing an unmethylated CpG motif(s) (17). The DNA binding activity of CGBP was demonstrated to reside within amino acids 106–345, a protein fragment that contains a CXXC domain. This domain is found in several DNA-binding proteins including HRX, MLL-2, DNMT1, and MBD1 (17–19). The studies described here establish that the CXXC domain is responsible for the unique DNA binding specificity of CGBP and define the preferred DNA binding site of this transcriptional activator.

The presence of the CXXC domain in a number of proteins that bind to CpG motifs is intriguing, although the function of this domain had not been clearly established. The CXXC domain resides within a fragment of DNMT1 that inhibits de novo methylation activity (25) and interacts with HDAC1 (26), and it has been reported to function as a transcriptional repression domain within the HRX protein (27, 28). With the exception of CGBP, proteins that contain a CXXC domain additionally contain other distinct DNA binding domains such as the methyl binding domain, AT-hooks, and zinc fingers (19, 29–32). Furthermore, the CXXC domain is dispensable for the DNA binding activity exhibited by DNMT1 and MBD1 (19, 25).

The studies reported here demonstrate that the minimal DNA binding domain of CGBP resides within a 60-amino acid domain (amino acids 162–221) that includes the CXXC domain. An intact CXXC domain is essential for DNA binding activity, as mutation to alanine of either of two conserved cysteine residues within the CXXC domain ablates DNA binding activity. Consistent with a requirement for a defined arrangement of highly conserved cysteine residues, CGBP requires the presence of Zn\(^{2+}\) for DNA binding activity. The minimal CXXC DNA binding domain of CGBP exhibits a DNA binding specificity for elements containing an unmethylated CpG dinucleotide.

Shortly after our description of the cloning of CGBP, Fujino et al. (33) independently report the cloning of this factor (denoted PCX1 in that report). They reported this factor as a regulator of the interstitial collagenase gene, MMP-1, and determined that a fragment containing amino acids 144–231, which includes the CXXC domain, exhibits DNA binding activity. Surprisingly, the MMP-1 promoter element used in this
one-hybrid screen lacks a CpG motif. However, linkers added to the end of the promoter fragment introduced CpG dinucleotides, likely explaining the paradoxical binding affinity of CGBP for this promoter element. Consistent with the determined binding specificity of CGBP (17), the binding of CGBP to the MMP-1 promoter element was not disrupted by an unrelated oligonucleotide that lacked a CpG motif but was disrupted by a mutated version of the MMP-1 promoter element that retained the linker CpG dinucleotides (33). Hence, CGBP does not appear to be an authentic regulator of the MMP-1 promoter.

Given our findings that the CXXC domain of CGBP exhibits affinity for unmethylated CpG motifs, it will be of interest to determine in more detail the structural requirements for this unique DNA binding specificity. These results also highlight the importance of additional studies on the contribution of the CXXC domain to the DNA binding activity of factors such as DNMT1, MBD1, HRX, and LXL-2 that contain additional distinct DNA binding domains (17–19). Interestingly, Fujita et al. (34) recently report that an alternatively spliced form of MBD1 binds either methylated or unmethylated promoters and attributes the ability to bind unmethylated DNA to the presence of a third copy of the CXXC domain. Interestingly, the CXXC domain from CGBP exhibits the highest degree of sequence homology (60% identity) to this alternatively utilized CXXC domain of MBD1.

Inspection of ligand-selected CGBP binding sites reveals a consensus sequence of (A/C)CpG(A/C). As expected, all recovered oligonucleotides contain at least one CpG dinucleotide, and introduction of mutations that remove the CpG motif(s) ablate the affinity of CGBP for these sequences. Introduction of mutations that either alter the sequence flanking the CpG dinucleotide or reduce the number of CpG motifs leads to a reduction in the affinity of CGBP. These results support the consensus CGBP binding site deduced from inspection of ligand-selected sequences recovered from a pool of degenerate oligonucleotides. Interestingly, in 20 of the 35 recovered sequences containing two CpG dinucleotides, these motifs are spaced 12–16 base pairs apart. Mutation of individual CpG motifs reduces the affinity of CGBP. These results suggest that CGBP exhibits greater affinity for clustered regions of CpG motifs, as found in CpG islands.

The studies reported here establish that the CXXC domain is responsible for the observed binding specificity of CGBP for unmethylated Cpg motifs and provide fundamental information regarding the structural basis for the unique DNA binding specificity of CGBP. These results should aid in future efforts to identify authentic target genes of CGBP action in vivo as well as more detailed analyses of the function of CXXC within other DNA-binding proteins.

REFERENCES

1. Bird, A. P. (1987) Trends Genet. 3, 342–347
2. Walsh, C. P., and Bestor, T. H. (1999) Genes Dev. 13, 26–34
3. Kafri, T., Ariel, M., Brundie, M., Shemer, R., Urven, L., McCarron, J., Cedar, H., and Razin, A. (1997) Mol. Cell. Biol. 17, 705–714
4. Tilghman, S. M. (1999) Cell 96, 185–193
5. Pfeifer, G. P., Tanguay, R. L., Steigerwald, S. D., and Riggs, A. D. (1990) Genes Dev. 4, 1277–1287
6. Okano, M., Bell, D. W., Haber, D. A., and Li, E. (1997) Genes Dev. 11, 247–257
7. Li, E., Bestor, T. H., and Jaenisch, R. (1992) Cell 69, 915–926
8. Xu, G.-L., Bestor, T. H., Bourc’his, D., Hsieh, C.-L., Tommerup, N., Bugge, M., Holten, M., Gu, X., Russos, J. J., and Viegas-Pequignot, E. (1999) Nature 402, 187–191
9. Amir, R. E., Van den Veyver, I. B., Man, M., Tran, C. Q., Francke, U., and Zoghbi, H. Y. (1999) Nat. Genet. 23, 185–188
10. Baylin, S. B., and Herman, J. G. (2000) Trends Genet. 16, 168–173
11. Hendrich, B., and Bird, A. (1998) Mol. Cell. Biol. 18, 6538–6547
12. Ng, H.-H., Zhang, Y., Hendrich, B., Johnson, C. A., Turner, B. M., Erdjument-Bromage, H., Tempst, P., Tenenberg, D., and Bird, A. (1999) Nat. Genet. 23, 568–572
13. Wade, P. A., Geoganne, A., Jones, P. L., Ballestar, E., Aubry, F., and Wolff, A. P. (1999) Nat. Genet. 23, 62–66
14. Macleod, D., Charlton, J., Mullins, J., and Bird, A. P. (1994) Genes Dev. 8, 2192–2202
15. Brandeis, M., Frank, D., Keshet, I., Siegfried, Z., Mendelsohn, M., Nemes, A., Temper, V., Razin, A., and Cedar, H. (1994) Nature 371, 435–438
16. Marin, M., Karis, A., Visser, P., Grossfeld, V., and Philipsen, S. (1997) Cell 89, 619–628
17. Voo, K. S., Carlone, D. L., Jacobsen, B. M., Flodin, A., and Skalnik, D. G. (2000) Mol. Cell. Biol. 20, 2108–2121
18. Heutinger, D. G., Chin, S.-P., Mulieris, M., Batley, S. J., Collins, V. P., Wiedemann, L., Aparicio, S., and Caldas, C. (1999) Oncogene 18, 7975–7984
19. Cross, S. H., Meehan, R. R., Nan, X., and Bird, A. (1997) Nat. Genet. 16, 256–259
20. Laemmli, U. K. (1970) Nature 227, 680–685
21. Sambrook, J., Fuchs, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 6.46–6.48, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Hagman, J., Gotch, M. J., Lin, H., and Grosschedl, R. (1995) EMBO J. 14, 2967–2976
23. Mavrothalassitis, G., Beal, G., and Papas, T. S. (1990) DNA Cell Biol. 9, 783–788
24. Sambrook, J., Fuchs, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 6.46–6.48, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Hagman, J., Gotch, M. J., Lin, H., and Grosschedl, R. (1995) EMBO J. 14, 2967–2976
26. Mavrothalassitis, G., Beal, G., and Papas, T. S. (1990) DNA Cell Biol. 9, 783–788
27. Zelenszki-Ko, N., Harden, A. M., and Bowley, J. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10610–10614
28. Prasad, R., Yano, T., Sorio, C., Nakamura, T., Rappaport, R., Gu, Y., Leshkowitz, D., Croce, C. M., and Canaani, E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 12160–12164
29. Bestor, T., Laudano, A., Mattaliano, R., and Ingram, V. (1988) J. Mol. Biol. 203, 971–983
30. FitzGerald, K. T., and Diaz, M. O. (1999) Genomics 59, 187–192
31. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C. M., and Canaani, E. (1999) Cell 71, 701–708
32. Ma, Q., Alder, H., Nelson, K. K., Chatterjee, D., Gu, Y., Nakamura, T., Canaani, E., Croce, C. M., Siracusa, L. D., and Buchberg, A. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 89, 6350–6354
33. Fujita, N., Hasagawa, M., Shihiya, S., Kato, T., Imai, S., Imai, M., and Takano, T. (2000) Biochem. Biophys. Res. Commun. 271, 305–310
34. Fujita, N., Shimotake, N., Ohki, T., Chiba, T., Saya, H., Shirakawa, M., and Nakao, M. (2000) Mol. Cell. Biol. 20, 5107–5118
35. Fujita, N., Takebayashi, S.-I., Okamura, K., Kudo, S., Chiba, T., Saya, H., and Nakao, M. (1999) Mol. Cell. Biol. 19, 6415–6426
