Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8891–8895, October 1993
Medical Sciences

Expression of an exogenous eukaryotic DNA methyltransferase gene induces transformation of NIH 3T3 cells

JIANJUN WU*†, JEAN-PIERRE ISSA*, JAMES HERMAN*, DOUGLAS E. BASSETT, JR.†, BARRY D. NELKIN*, AND STEPHEN B. BAYLIN*‡

*Oncology Center, ‡Department of Medicine and †Predoctoral Program in Human Genetics and Molecular Biology, The Johns Hopkins University Medical Institutions, Baltimore, MD 21231

Communicated by Bert Vogelstein, June 7, 1993 (received for review September 14, 1992)

ABSTRACT **Abnormal regional increases in DNA methylation, which have potential for causing gene inactivation and chromosomal instability, are consistently found in immortalized and tumorigenic cells. Increased DNA methyltransferase activity, which is also a characteristic of such cells, is a candidate to mediate these abnormal DNA methylation patterns. We now show that, in NIH 3T3 mouse fibroblasts, constitutive overexpression of an exogenous mouse DNA methyltransferase gene results in a marked increase in overall DNA methylation which is accompanied by tumorigenic transformation. These transformation changes can also be elicited by dexamethasone-inducible expression of an exogenous DNA methyltransferase gene. Our findings provide strong evidence that the increase in DNA methyltransferase activity associated with tumor progression could be a key step in carcinogenesis and provide a model system that can be used to further study this possibility.**

Abnormal patterns of DNA methylation are a consistent molecular feature of immortalized and neoplastic cells (for review, see refs. 1 and 2). An important aspect of these changes is the abnormal *de novo* methylation (2–4) of normally unmethylated clusters of cytosine–guanosine dinucleotides, or "CpG islands," which are usually located in the 5′ region of genes (5). This abnormal methylation has been associated with changes of chromatin structure (3, 4) that can inhibit gene expression (6–9). DNA methylation could also result in changes in DNA sequences, since methylated cytosine is a highly mutable base in the eukaryotic genome (10–12).

One potential mechanism for establishing abnormal patterns of DNA methylation in neoplastic cells involves the increases in DNA methyltransferase (DNA MTase) activity that we (13) and others (14) have associated with tumor evolution. Expression of the MTase gene which encodes the enzyme that catalyzes cytosine methylation at CpG sites is abnormally high in tumor cells (13, 14) and increases throughout the progression stages of human colon cancer (13). Recently, it has also been suggested that DNA MTase might potentially act directly as an endogenous mutagen by causing enzymatic deamination of cytosine at a very high frequency (15). To evaluate the potential consequences of increased DNA MTase activity in neoplastic cells, we have now inserted an exogenous mouse DNA MTase gene in NIH 3T3 cells. We find that overexpression of this gene results in a marked increase of overall DNA methylation accompanied by transformation of the cells as manifested by loss of contact inhibition, acquisition of ability to grow in soft agar, and appearance of tumorigenic capacity in nude mice. These results strongly indicate that the increased expression of the DNA MTase gene seen in neoplastic cells could constitute a key mechanism underlying tumor progression.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Culture. NIH 3T3 cells were grown in Dulbecco's modified Eagle's medium (Paragon Biotechnology, Baltimore, MD) containing glucose (4.5 mg/ml), sodium pyruvate (0.11 mg/ml), and supplemented with 10% bovine calf serum (HyClone) and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml) (GIBCO).

Plasmid Constructs and Transfection. The full-length cDNA for the mouse DNA MTase gene (16) was placed in both sense and antisense orientations into the *Bam*HI site of the expression vector pCMV-Neo-Bam, which has been successfully used to express exogenous genes in eukaryotic cells (17, 18). The same vector without insert was used as control. Ten micrograms of each plasmid construct was transfected into NIH 3T3 fibroblast cell lines by lipid-mediated gene transfer (19) using 40 μg of Lipofectin (BRL, catalogue no. 8292SA) as suggested by the manufacturer. Multiple G418-resistant colonies were randomly picked 20 days after transfection for each construct and expanded for further study.

Reverse Transcription (RT)–PCR Detection of the Exogenous DNA MTase Gene. We used several strategies, each employing previously utilized RT–PCR analyses (13), to detect both exogenous and endogenous DNA MTase transcripts. For all cell types studied, 1 μg of total RNA was used and control tubes with omission of RNA or reverse transcriptase were included in each assay. The initial reverse transcriptase step was carried out for 1 hr at 42°C. The PCR products were run in 2% agarose gels and transferred to nylon membranes and hybridized (13) with the full-length insert for the mouse DNA MTase gene.

DNA MTase Enzyme Assay. Cells were harvested by trypsinization, washed twice in phosphate-buffered saline, and suspended (3×10^5 cells in 100 μl) in lysis buffer [50 mM Tris·HCl, pH 7.8/1 mM EDTA, pH 8.0/1 mM dithiothreitol/0.01% (wt/vol) NaN_3/10% (vol/vol) glycerol/1% (vol/vol) Tween 80 containing phenylmethanesulfonyl fluoride at 60 μg/ml]. The cells were then lysed by four cycles of freezing at −70°C and thawing at 37°C. Protein concentration in the lysate was determined by the Bradford assay, and the lysate was diluted to 0.5 μg of protein in 15 μl of lysis buffer. DNA MTase activity was assayed by the procedure of Adams *et al.* (20). All assays were performed by one investigator (J.-P.I.) who was blinded to the identity of the specimens.

DNA Methylation Level. A modification of the methyl-accepting assay (21) was used to determine the methylation status of DNA isolated from the transfected and control 3T3 cells. DNA (200 ng) was incubated with 4 units of M.*Sss* I

Abbreviations: MTase, methyltransferase; RT, reverse transcription.

CpG methylase (New England Biolabs) in the presence of 1.5 μM *S*-adenosyl-L-[methyl-^{3}H]methionine (80–85 Ci/mmol; Amersham, TRK 581) and 1.5 μM nonradioactive *S*-adenosylmethionine (New England Biolabs). The reaction mixtures (20 μl), in a buffer containing 10 mM Tris·HCl (pH 7.9), 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol were incubated at 37°C for 4 hr. The reactions were stopped by adding 5 μl of 2.5 mM nonradioactive *S*-adenosylmethionine. The reaction mixtures were then spotted on GF/C 2.4-cm^2 Whatman filter discs, which were air dried for 15 min, washed with 6 ml of 5% (wt/vol) trichloroacetic acid and 6 ml of 70% (vol/vol) ethanol, and placed in a scintillation vial containing 10 ml of Econofluor (NEN) for assay of radioactivity in a Beckman liquid scintillation counter. Reactions without either DNA or enzyme added were included as background controls, and counts in these samples never exceeded 2–5% of those for the test samples. All samples were done in duplicate and values were obtained as dpm/ng of DNA.

RESULTS

Morphology and Growth Characteristics of the Transfected Cells. Both sense and antisense transfectants showed marked phenotypic changes in growth characteristics and morphology. Ten of 11 individual sense DNA MTase transfectant clones (Fig. 1*A*) were more spindle-shaped and had increased cytoplasmic granularity compared with parent cells and cells transfected with the vector alone. Before and after reaching confluency, these sense clones tended to grow in disarray as compared with the linear array of control cells (Fig. 1*C*). They exhibited a decrease in contact inhibition and grew to a higher density (Fig. 1*D* and Table 1). Interestingly, the only sense clone not showing the above characteristics was one shown below to have a rearranged DNA MTase gene insert. In sharp contrast to the phenotype of the sense DNA MTase clones, 5 of 8 antisense transfectants analyzed, as compared with control cells, had very flat morphology, had increased cell size with a cobblestone shape, and grew to a lower saturation density than the sense clones (Fig. 1 *B* and *D*; Table 1).

Expression of the Transfected Constructs. As analyzed by Southern blot analysis (data not shown), 10 of 11 sense clones had integrated a full-length exogenous DNA MTase gene. The 11th sense clone was rearranged within the gene insert. The expression of exogenous genes was assayed by RT–PCR. To detect the far 5′ region of mRNA coming from the transfected constructs of the sense-orientation DNA MTase gene, we used an upstream primer within the transcribed region of the cytomegalovirus promoter (Fig. 2*A*, primer 1) and a downstream primer from the DNA MTase insert (primer 2). For analysis of the 5′ mRNA for antisense DNA MTase transfectants, the same upstream primer described above was used, and the downstream primer corresponded to DNA MTase sequence 4802–4822 (not shown). This assay for the far 5′ region of the exogenous genes showed that the predicted spliced RNA products were present for all sense- and antisense-transfected clones (data not shown).

To verify that the full-length DNA MTase gene was expressed in sense DNA MTase-transfected clones, and to compare its expression with the endogenous DNA MTase expression, we coamplified by RT–PCR the 3′ end of the endogenous and exogenous mRNAs. A 412-bp product for endogenous DNA MTase transcripts could be detected, by pairing primers 3 and 4 (Fig. 2). A distinct exogenous transcript (440 bp) could be seen, by pairing primers 3 and 5 (Fig. 2*A*), in all but the one sense clone containing the rearranged MTase insert (Fig. 2*B*, lane 7). These data, when paired with results of the 5′-specific RT–PCR, indicate that this rearranged clone cannot express a full-length DNA MTase transcript. In virtually all other sense clones, in three separate RT–PCR assays, the exogenous DNA MTase gene transcripts were more abundant than the endogenous transcripts (Fig. 2*B*) by an average of at least 2.2-fold as estimated by densitometric analysis of hybridization signals.

DNA MTase Activity in the Transfected Cells. We next compared the DNA MTase enzyme activity in sense, antisense, and vector-alone clones. Since expression of the endogenous DNA MTase gene is dynamically regulated as a function of cell growth, we first analyzed randomly selected transfectant clones for this relationship. Interestingly, even a sense-transfected clone which had predominantly exogenous MTase RNA transcripts showed the same dynamic regulation of enzyme activity as seen in a 3T3 clone transfected with the vector alone or one clone with the antisense construct. In each case, enzyme activity was distinctly highest in early logarithmic-phase growth and low in cells at confluency (Fig. 3*A*). However, in the sense clones, mean DNA MTase activity was increased, in early growth, 2-fold over levels in the control or antisense clones. This increase persisted, albeit at a lower level, throughout the growth curve. This finding indicates that DNA MTase transcripts expressed from a constitutive cytomegalovirus promoter may still be regulated

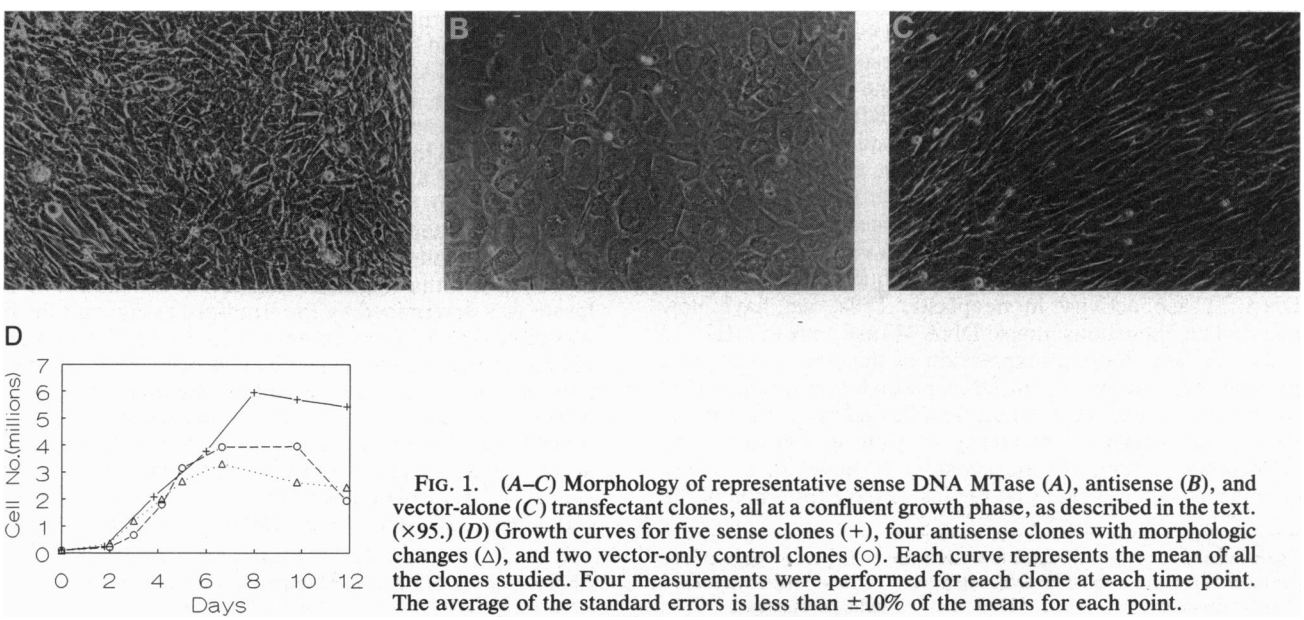

FIG. 1. (*A–C*) Morphology of representative sense DNA MTase (*A*), antisense (*B*), and vector-alone (*C*) transfectant clones, all at a confluent growth phase, as described in the text. (×95.) (*D*) Growth curves for five sense clones (+), four antisense clones with morphologic changes (△), and two vector-only control clones (○). Each curve represents the mean of all the clones studied. Four measurements were performed for each clone at each time point. The average of the standard errors is less than ±10% of the means for each point.

Table 1. Characteristics of transfected NIH 3T3 cell lines

Clone type	DNA MTase enzyme activity, dpm/μg of protein	DNA methylation level, % control	Saturation density, cell no. × 10^{-6} per dish	Soft-agar cloning, no. of colonies per dish	Tumorigenicity, tumors/injections	
					Day 20	Day 30
Sense	6918 ± 954 (9)	142 ± 5.2 (7)	6.17 ± 0.15 (5)	310.0 ± 53.0 (9)	32/48 (9)	40/48 (9)
AS−	4057 ± 644 (3)	113 ± 5.1 (3)	4.51 ± 0.29 (3)	11.0 ± 8.6 (3)	4/12 (3)	10/12 (3)
AS+	1510 ± 165 (5)	36 ± 7.5 (4)	3.24 ± 0.17 (4)	6.5 ± 3.2 (4)	1/20 (5)*	1/20 (5)*
Control	3457 ± 460 (7)	100 ± 5.8 (4)	3.93 ± 0.25 (2)	7.0 ± 4.1 (4)	1/28 (7)†	0/28 (7)†

The values for enzyme activity, methylation levels, saturation density, and soft-agar cloning are presented as mean ± SEM. Values in parentheses represent numbers of individual clones analyzed. Sense, clones transfected with DNA MTase in sense orientation; AS−, antisense construct-transfected clones which do not have morphology change; AS+, antisense construct transfected clones with morphology changes; control, colonies transfected with vector alone.

*One mouse developed a 0.4-cm mass at day 20 which regressed at day 30. Another mouse developed a tumor of 0.4 cm at day 30 which persisted without further growth.

†The 0.4-cm mass seen in one mouse by day 20 had regressed at day 30.

as a function of cell growth. Such regulation fits with data of Szyf et al. (22) showing that the growth-related dynamics of endogenous DNA MTase gene expression are mediated by posttranscriptional control.

With the above data in mind, we checked all transfected clones at subconfluence to assess expression of the inserted DNA MTase gene, at the protein level. We picked this cell stage for convenience of cell harvest even though, given the data discussed directly above, we would expect that differences between clones might be somewhat reduced at this growth point. Nevertheless, the sense clones had overall activity distinctly higher ($P < 0.01$) than the vector control clones, with an average 2-fold, and maximum 3-fold, increase (Fig. 3B). These average increases agree well with those of our previous RNA analyses indicating similar increases of exogenous over endogenous DNA MTase RNA transcripts (Fig. 2B). Moreover, we observed for each sense clone a tight relationship ($r = 0.91$; $P < 0.001$) for level of DNA MTase enzyme activity versus the exogenous mRNA expression levels determined by densitometry (Fig. 3C). Importantly, no significant correlation was noticed between the endogenous DNA MTase RNA levels and the enzyme activities (Fig. 3C, $r = 0.47$; $P = 0.20$). These data provide firm evidence that the increased DNA MTase activity in the sense DNA MTase-transfected clones was derived from the expression of the exogenous gene.

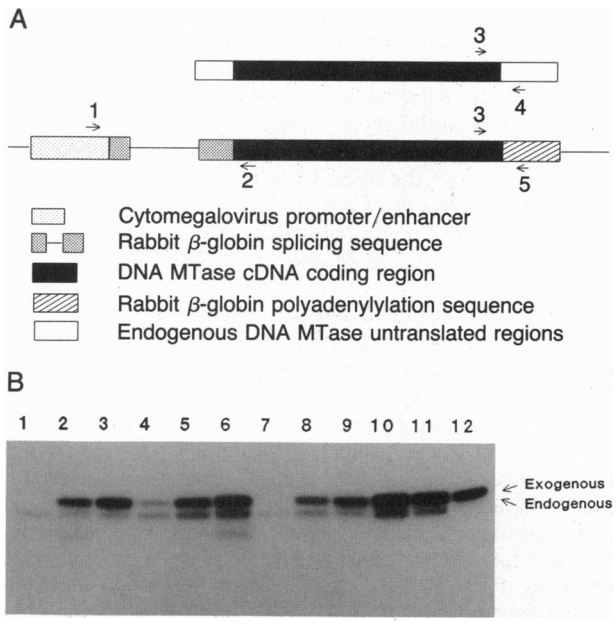

FIG. 2. (A) Schematic drawing of the expression construct for the cytomegalovirus promoter-driven sense-orientation DNA MTase gene (lower bar) and corresponding regions of the endogenous transcript (upper bar). The RT–PCR primers for detecting both the endogenous and exogenous DNA MTase transcripts in transfected cells (see text for details) were primer 1, 5′-TTGACCTCCATAGAAGACACCG-3′; primer 2, 5′-TTAAGAGGGACTTGACTTTAGCC-3′; primer 3, 5′-GCAACATCCTGGACAGAC-3′; primer 4, 5′-AGTCTCACTTGCCACCTG-3′; and primer 5, 5′-ATTGGCCACACCAGCCAC-3′. (B) Hybridization signal for amplification products from RNA of sense-orientation transfectant clones, for the 3′ region of the endogenous and exogenous DNA MTase transcripts. The top band in each lane is the exogenous product (440 bp) and the lower band represents endogenous-specific product (412 bp). Lane 1, cells transfected with vector only; lanes 2–12, individual sense DNA MTase-transfected clones. Lane 7 represents the clone with the rearranged DNA MTase insert (see text for details). In lane 12, the endogenous signal for this clone could be clearly detected with a longer exposure.

In sharp contrast to the above data, the five antisense clones with morphologic changes had DNA MTase activity values lower than the lowest value for vector-alone cells. However, the three morphologically unchanged clones actually had values in the high vector-alone range (Fig. 3B and Table 1).

Changes in DNA Methylation in the Transfected Cells. We next assessed whether expression of the inserted sense and antisense DNA MTase genes caused changes in the methylation status of DNA. For this, we used a DNA methyl-accepting assay in which the capacity of cellular DNA to undergo *in vitro* methylation is inversely proportional to its endogenous methylation level. We found striking differences between the transfected cells and vector-alone clones which virtually paralleled the previously described changes in DNA MTase activity. The sense DNA MTase-transfected cells had, on average, as compared to vector-alone controls, a 42% decrease in methyl-accepting capacity, indicating marked hypermethylation (Fig. 3D). These data indicate that the transfected sense DNA MTase gene produced an active enzyme capable of reaching DNA targets and causing profound overall changes in DNA methylation. In contrast, the antisense DNA MTase transfectant clones, which had a marked morphologic change, had a 64% increase in methyl-accepting capacity, indicating marked undermethylation (Fig. 3D). However, the three antisense clones which did not have decreased DNA MTase activity had DNA methylation levels in the high vector-alone-clone range (Fig. 3D). These data indicate that DNA methylation levels reflected the antisense activity of the transfected gene and that the three clones which did not undergo morphologic change did not have this antisense effect.

Clonogenicity of the Transfected Cells. The phenotypic changes of the sense DNA MTase-transfected clones, especially the loss of contact inhibition, suggested to us that these cells might have a high oncogenic potential. This was initially

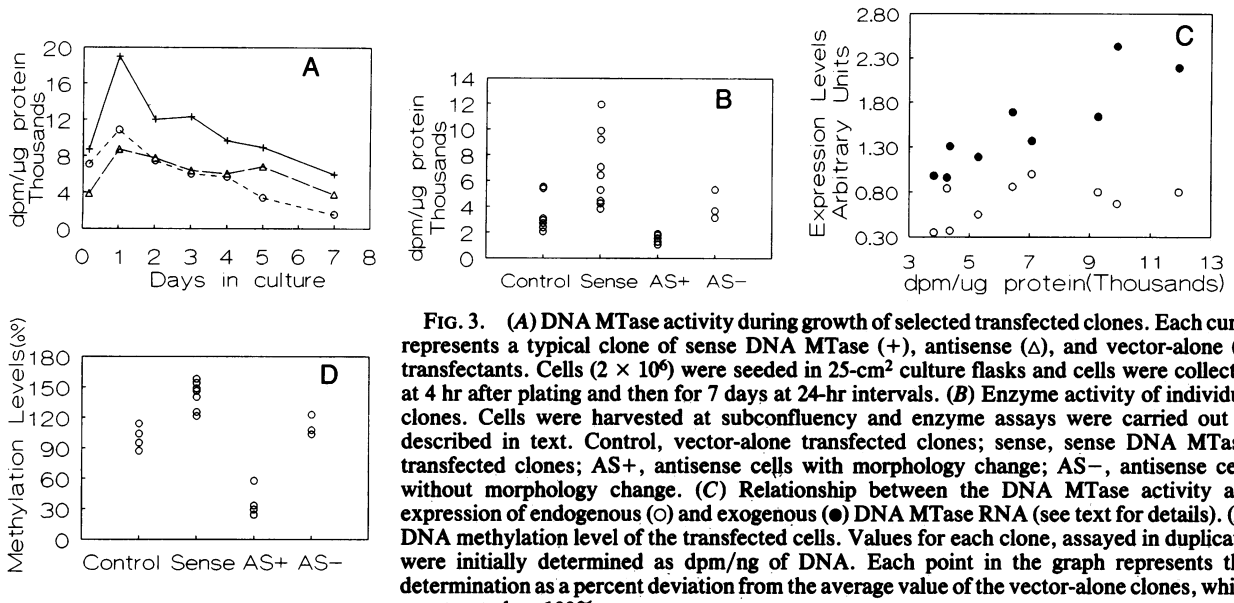

FIG. 3. (A) DNA MTase activity during growth of selected transfected clones. Each curve represents a typical clone of sense DNA MTase (+), antisense (Δ), and vector-alone (○) transfectants. Cells (2×10^6) were seeded in 25-cm^2 culture flasks and cells were collected at 4 hr after plating and then for 7 days at 24-hr intervals. (B) Enzyme activity of individual clones. Cells were harvested at subconfluency and enzyme assays were carried out as described in text. Control, vector-alone transfected clones; sense, sense DNA MTase-transfected clones; AS+, antisense cells with morphology change; AS−, antisense cells without morphology change. (C) Relationship between the DNA MTase activity and expression of endogenous (○) and exogenous (●) DNA MTase RNA (see text for details). (D) DNA methylation level of the transfected cells. Values for each clone, assayed in duplicate, were initially determined as dpm/ng of DNA. Each point in the graph represents this determination as a percent deviation from the average value of the vector-alone clones, which was treated as 100%.

studied by assessing ability of the cells to be cloned in soft agar. By day 20, 10 of 11 sense DNA MTase-transfected clones showed clonogenicity (3.1%) in soft agar (Table 1). The only clone that failed to grow in soft agar was, again, the rearranged one which lacked a full-length exogenous DNA MTase transcript. In contrast, 4 vector control and 7 antisense transfectants were not clonogenic (0.07–0.1%; Table 1).

The above cloning data suggested that insertion and expression of the sense DNA MTase gene, and resultant DNA hypermethylation, directly resulted in progression of 3T3 cells to a clonogenic state. To rule out other factors, such as high background clonogenicity of the initial cells studied and effects of insertional events of the expression vectors not involving DNA methylation, we transfected earlier passages of 3T3 cells with a sense-oriented DNA MTase gene behind the dexamethasone-inducible mouse mammary tumor virus promoter in the expression vector pMAM-Neo (23). Cells from G418-selected clones were exposed for 5 weeks, including 20 days of soft-agar cloning, to 1 μM dexamethasone. Those which had induced expression of the inserted DNA MTase gene by RT–PCR assay, as compared with cells from the same clones not exposed to dexamethasone, showed the same striking increase in clonogenicity as the clones transfected with the cytomegalovirus promoter-driven DNA MTase gene (Table 2). These results directly demonstrate the active role of exogenous DNA MTase activity in causing increased clonogenicity of 3T3 cells.

Tumorigenicity of the Transfected Cells. The tumorigenicity of each transfectant cell line was directly tested by subcutaneously injecting groups of four to eight nude athymic mice per clone. The sense DNA MTase transfected clones were the only ones to consistently form tumors (Table 1). At day 20, 67% of the animals injected with these clones developed tumors (0.5–1.3 cm in diameter). At day 30, tumors were present in 83% of the animals (0.4–2.0 cm in diameter). The clone with the rearranged DNA MTase insert was not tumorigenic for any of the mice, nor were the vector-alone controls or the antisense clones which had a morphology change.

To assess to what extent the increased levels of DNA MTase activity in the sense clones might directly contribute to tumor growth, we analyzed the relationship between enzyme activity of the injected cells and final tumor size. A correlation coefficient of $r = 0.75$ ($P < 0.02$) was obtained, suggesting a direct relationship between level of expression of the transfected DNA MTase gene and degree of tumor growth.

One puzzling result in our study concerns the three antisense-transfected clones that did not manifest obvious morphologic changes and which actually maintained levels of DNA MTase activity, overall DNA methylation, and saturation density slightly higher than the average for the vector-alone control clones (Table 1). These cells were tumorigenic in nude mice. However, the tumors grew more slowly than those in the above sense-transfected clones. While we cannot yet explain the tumorigenicity of these clones, we suggest that the cells may have escaped the initial antisense effect by increasing the endogenous DNA MTase activity and becoming transformed over time.

DISCUSSION

This study reveals that constitutive or induced overexpression of an exogenous eukaryotic DNA MTase gene results in transformation of NIH 3T3 cells including decreased contact

Table 2. Soft-agar cloning data for nine clones transfected with sense DNA MTase under control of a dexamethasone-inducible mouse mammary tumor virus promoter

	Without dexamethasone	With 1 μM dexamethasone	
		RNA$^-$	RNA$^+$
No. of independent clones with cloning efficiency ≥ 0.5%	0/9	0/3	6/6
Average no. of colonies per dish (mean ± SEM)	11 ± 3.2*	18.3 ± 3.1†	215.7 ± 45.2

*$P < 0.01$, by Student's t test, when compared with values for the clones that were exposed to 1 μM dexamethasone and that had, by RT–PCR, clear evidence of induced exogenous transcripts (RNA$^+$).
†$P < 0.05$, when these clones, which had no evidence of exogenous DNA MTase transcripts (RNA$^-$), even after dexamethasone exposure, are compared with the RNA$^+$ clones.

inhibition, increased ability to clone in soft agar, and acquisition of ability to form tumors in nude mice. The tight correlation in our data between these induced phenotypic features and levels of exogenous mRNA from the inserted DNA MTase gene, as well as changes in DNA MTase activity and changes in overall DNA methylation, supports a direct role for the exogenous expressed gene in causing tumorigenic progression of the cells.

The findings in our experimental model can now be viewed in context of the known increases in DNA MTase activity which accompany tumor progression (13, 14). Our observations provide experimental evidence that this change in neoplastic cells could play a critical role in the evolution of neoplasms. As previously discussed, increased expression of the DNA MTase gene, as represented by steady-state mRNA and enzyme activity levels, appears to occur progressively over increasing stages of colon tumors (13, 24) and is characteristic of cultured rodent and human tumors of multiple types (13, 14). The increases in DNA MTase activity produced in our transfected clones are in the range, 2- to 3-fold, that we have recently found for increasing stages of colon carcinoma (24). While these increments may seem small, our results now indicate that such perturbations of the endogenously well-regulated control of DNA MTase activity, especially when established relatively rapidly in a cell culture setting, can result in profound overall changes in DNA methylation, as shown in Fig. 2*D*. These methylation changes, in turn, appear to have great potential for altering cell phenotype.

The precise mechanism by which increased DNA MTase activity can cause tumor progression remains to be determined. However, another consistent alteration of DNA methylation occurring during tumor progression, and again, characteristic of established rodent and human neoplastic cells (1–4, 25–27), may well explain our results. Regional increases in DNA methylation, which could affect chromatin structure and gene function, are increasingly being associated with chromosome loci that consistently undergo loss of tumor-suppressor gene function in immortalized or neoplastic human and mouse cells (2, 4, 25, 27). These changes, like increases in DNA MTase activity, appear early in progression of cancers such as colon carcinoma (27) and often involve normally unmethylated clusters of CpG sites in the 5′ regulatory regions of genes (2). Methylation of such CpG sites has been shown to inhibit gene transcription (6–9). Such a change has been correlated with a region of chromosome instability and with decreased expression of the gene responsible for the inherited mental retardation disease, fragile X syndrome (28–30). Also, CpG dinucleotides are mutational hotspots, since 5-methylcytidine residues are highly mutable and engage in C → T transitions through deamination (10–12). Increased expression of the DNA MTase gene may also cause high mutation rates at CpG sites by direct enzymatic deamination of cytosine (15). Abnormal *de novo* methylation in the genome of neoplastic cells could then contribute to loss of tumor-suppressor gene function by direct silencing of gene expression, by causing gene mutations, or by contributing to structural alterations of chromosomes (for review, see refs. 1 and 2).

In summary, our current results indicate that the increased DNA MTase gene expression detected in human tumors, or tumor precursor cells, could constitute a key mechanism underlying the evolution of neoplasia. This change could be a central step, induced by multiple different tumorigenic stimuli, leading to immortalization of normal cells and subsequent transformation during tumor progression. Overexpression of an exogenous DNA MTase gene, especially in human cell precursors to tumor types which consistently exhibit abnormal DNA methylation, appears to offer a model which should further clarify the precise relationships between abnormal DNA methylation, altered gene expression, genetic instability, and tumor evolution.

We thank Dr. Timothy Bestor for providing the full-length DNA MTase cDNA; Dr. Bert Vogelstein and Daniel Levy for providing the expression vector pCMV-Neo-Bam; Drs. Paul Celano, Paula Vertino, and Jonathan Simons for critical review of the manuscript and valuable suggestions; and Ms. Tammy Hess and Ms. Sandra Lund for administrative and secretarial assistance. These studies were supported by National Institutes of Health Grant CA43318 and by grants from the Tobacco Research Foundation and the Clayton Fund.

1. Jones, P. A. & Buckley, J. D. (1990) *Adv. Cancer Res.* **54**, 1–23.
2. Baylin, S. B., Makos, M., Wu, J. J., Yen, R.-W. C., de Bustros, A., Vertino, P. & Nelkin, B. D. (1991) *Cancer Cells* **3**, 383–390.
3. Antequera, F., Boyes, J. & Bird, A. P. (1990) *Cell* **62**, 503–514.
4. de Bustros, A., Nelkin, B. D., Silverman, A., Ehrlich, G., Poiesz, B. & Baylin, S. B. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5693–5697.
5. Bird, A. P. (1986) *Nature (London)* **321**, 209–213.
6. Keshet, I., Lieman-Hurwitz, J. & Cedar, H. (1986) *Cell* **44**, 535–543.
7. Tazi, J. & Bird, A. P. (1990) *Cell* **60**, 909–920.
8. Cedar, H. (1988) *Cell* **53**, 3–4.
9. Boyes, J. & Bird, A. P. (1991) *Cell* **64**, 1123–1134.
10. Bird, A. P. (1980) *Nucleic Acids Res.* **8**, 1499–1504.
11. Baker, D., Schafer, M. & White, R. (1984) *Cell* **36**, 131–138.
12. Cooper, O. & Youssoufian, H. (1988) *Hum. Genet.* **78**, 151–155.
13. El-Deiry, W. S., Nelkin, B. D., Celano, P., Yen, R.-W. C., Falco, J. P., Hamilton, S. R. & Baylin, S. B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3470–3474.
14. Kautainien, T. L. & Jones, P. A. (1986) *J. Biol. Chem.* **261**, 1594–1598.
15. Shen, J.-C., Rideout, W. M., III, & Jones, P. A. (1992) *Cell* **71**, 1073–1080.
16. Bestor, T., Laudano, A., Mattaliano, R. & Ingram, V. (1988) *J. Mol. Biol.* **203**, 971–983.
17. Kavasuyama, H., Tokyama, N. & Tada, T. (1989) *J. Exp. Med.* **169**, 13–25.
18. Baker, S. J., Markowitz, S., Fearon, F. R., Wilson, V. & Vogelstein, B. (1990) *Science* **249**, 912–915.
19. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. & Danielsen, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7413–7417.
20. Adams, R. L. P., Rinaldi, A. & Seivwright, C. (1991) *J. Biochem. Biophys. Methods* **22**, 19–22.
21. Bestor, T. & Ingram, V. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5559–5563.
22. Szyf, M., Bozovic, V. & Tanigawa, G. (1991) *J. Biol. Chem.* **266**, 10027–10030.
23. Sardet, C., Franchi, A. & Pousségur, J. (1989) *Cell* **56**, 271–280.
24. Issa, J.-P., Vertino, P. M., Wu, J., Sazawal, S., Celano, P., Nelkin, B. D., Hamilton, S. R. & Baylin, S. B. (1993) *J. Natl. Cancer Inst.* **85**, 1235–1240.
25. Baylin, S. B., Fearon, E. R., Vogelstein, B., de Bustros, A., Sharkis, S. J., Burke, P. J., Staal, S. P. & Nelkin, B. D. (1987) *Blood* **70**, 412–417.
26. Jones, P. A., Wolkowicz, M. J., Rideout, W. M., III, Gonzales, C. M., Marziasz, C. M., Coetzee, G. A. & Tapscott, S. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6117–6121.
27. Makos, M., Nelkin, B. D., Lerman, M. I., Latif, F., Zbar, B. & Baylin, S. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1929–1933.
28. Bell, M. V., Hirst, M. C., Nakahori, Y., MacKinnon, R. N., Roche, A., Flint, T. J., Jacobs, P. A., Tommerup, N., Tranebjaerg, L., Froster-Iskenius, U., Kerr, B., Turner, G., Lindenbaum, R. H., Winter, R., Pembrey, M., Thidodeau, S. & Davis, K. E. (1991) *Cell* **64**, 861–866.
29. Heitz, D., Rousseau, F., Devys, D., Saccone, S., Abderrahim, H., Le Paslier, D., Cohen, D., Vincent, A., Toniolo, D., Della Valle, G., Johnson, S., Schlessinger, D., Oberlé, I. & Mandel, J. L. (1991) *Science* **251**, 1236–1239.
30. Oberlé, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., Boue, J., Bertheas, M. F. & Mandel, J. L. (1991) *Science* **252**, 1097–1102.