Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5693–5697, August 1988
Medical Sciences

The short arm of chromosome 11 is a "hot spot" for hypermethylation in human neoplasia

ANDRÉE DE BUSTROS*, BARRY D. NELKIN*, ANN SILVERMAN*, GARTH EHRLICH†, BERNARD POIESZ†, AND STEPHEN B. BAYLIN*

*The Oncology Center, School of Medicine, The Johns Hopkins University, Baltimore, MD 21215; and †State University of New York Health Science Center, Syracuse, NY 13210

Communicated by John W. Littlefield, April 12, 1988

ABSTRACT **Inactivation of normally expressed genes may play a role in the formation and/or progression of human cancers. Methylation of cytosine in DNA could potentially participate in such alterations of gene expression. Abnormalities in DNA methylation are a consistent feature of human neoplasms, and we now show that these include not only previously recognized widespread genomic hypomethylation, but also regional increases in gene methylation. A hot spot for abnormal methylation of C + G-rich areas has been detected on the short arm of chromosome 11 in an area known to harbor tumor suppressor genes. This change occurs consistently in common forms of human cancer and appears early during the transformation of cells with viruses including members of the human T-cell leukemia (HTLV) family. Furthermore, in one chromosome 11 gene examined, calcitonin, the increased methylation in somatic tumor cells coincides with the presence of an "inactive" chromatin pattern in the transcriptional regulatory area. The increased regional DNA methylation demonstrated may then participate in or mark chromosomal changes associated with gene inactivation events that are central to the genesis and/or progression of human cancers.**

Loss of expression of "tumor suppressor" genes may be a fundamental process underlying formation and/or progression of many types of cancer (1–5). One event in DNA that may mark and/or participate in chromosomal events influencing such changes in gene transcription, and that is altered in human cancer, is DNA methylation. Decreased methylation of C+G-rich areas in the 5′ region of genes may be "permissive" but not sufficient for allowing gene expression (6, 7). In contrast, *in vitro* studies suggest that increased methylation in these same areas prevents gene transcription (8).

Studies of overall DNA methylation have revealed either no difference between normal and cancer cells or hypomethylation in cancer cells (9–11). Where specific genes have been studied, hypomethylation has been observed in human neoplasms (12–14). By contrast, Kautainien and Jones (15) recently reported increased activity of a C + G-specific methylase in cancer cells. This increase did not correlate with changes in overall DNA methylation and the authors suggested that unidentified localized hot spots for DNA hypermethylation, not reflected in the total DNA methylation pattern (15), might exist in cancer cells. In this regard, we recently found in human cancers a pattern of increased methylation in the 5′ region of the calcitonin gene (*CALC1*) (16, 17). This gene resides on the short arm of chromosome 11 (18, 19), the site of at least two "tumor suppressor" genes (4, 5). Clusters of genes in this area are conserved on other chromosomes in nonhuman species (20). We now show that this change in the *CALC1* gene is associated with a region of increased methylation on the short arm of chromosome 11, which coexists with widespread genomic hypomethylation. This *CALC1* gene hypermethylation occurs early in viral transformation and marks and/or participates in structural changes in the DNA regulatory region of the gene that would inactivate transcription. Regional DNA hypermethylation must then be considered as one potential mechanism for gene inactivation in human cancer.

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 Lines, DNA Preparation, and DNA Hybridization. Human cells studied consisted of lung cancer lines started in either our own laboratory (21) or that of Adi Gazdar (22), colon cancer lines (23), fibroblasts (24, 25), teratocarcinoma cells (26), and cord blood lymphocytes infected with the human T-cell lymphotropic virus (HTLV) type I (27). Fresh human T lymphocytes were infected with this virus as described (28). DNA and RNA extraction and Southern and RNA blot hybridizations were performed as described (16, 17).

Molecular Probes. The 5′ region *CALC1* genomic probe has been described (16, 17). Other probes for the short arm of chromosome 11 included an anonymous region, pADJ 762 (29), a full-length genomic probe of the *HRAS* gene, which spans 5′ and 3′ flanking regions (30–32), and a 5′ region genomic probe for catalase (*CAT*) (33). Probes for genes on other chromosomes included a full-length genomic probe of human *MYC* (34), an *Xho* I/*Nco* I 5′ fragment of a genomic probe for *FOS* (35, 36), a genomic probe for the transferrin receptor (37, 38), and 5′ region genomic probes for the *HLA-A2* (39) and *HLA-B7* (40) genes.

Assessment of CCGG Methylation Patterns. The numbers of CCGG sites methylated were assessed by digesting DNA with *Msp* I and *Hpa* II, which cut at the sequence CCGG. *Hpa* II will not cut when the internal cytosine is methylated (41). DNA (5 μg) was digested with either 10 units of *Msp* I per μg or 4 units of *Hpa* II per μg, electrophoresed, transferred to nylon filters (DuPont), and hybridized to selected ^{32}P-labeled DNA probes. Filters were used for multiple hybridizations and the previous probe was removed by boiling for 20 min in 0.1 × SSC/0.1% $NaDodSO_4$ (1 × SSC = 0.15 M NaCl/0.015 M sodium citrate).

Studies of *CALC1* Gene Chromatin Conformation. For identification of nuclease hypersensitive sites at the 5′ end of the *CALC1* gene, intact nuclei were digested with restriction endonucleases for known sites in this region. Intact nuclei were prepared as described by Sweet *et al.* (42) and suspended at 2 × 10^8 nuclei per ml of digestion buffer (20 mM Tris, pH 7.9/50 mM NaCl/3 mM $MgCl_2$/0.1 mM EGTA/1 mM 2-mercaptoethanol). After incubation for 1 hr at 37°C with increasing concentrations of *Msp* I (0, 45, 90, and 450 units/ml) or selected other restriction enzymes, samples

Abbreviation: HTLV, human T-cell lymphotropic virus.

were deproteinized, DNA was extracted, digested with *Bgl* II or *Bam*HI, electrophoresed, and hybridized to the 5′ *CALC1* gene probe.

RESULTS

Methylation Patterns of the *CALC1* Gene in Cultured Human Cancer Cells. Our initial studies of DNA from patient tumor samples indicated that hypermethylation of the *CALC1* gene was present in virtually all leukemias (17) and small cell lung cancers (16). Recently, we have studied 23 established lines of all types of lung cancers, 17 of gastrointestinal tumors, and 1 teratocarcinoma line and found that 83% (data not shown) contained the previously reported methylation abnormalities of the *CALC1* gene. As discussed later, this change is not induced by culture conditions alone.

Methylation Patterns of Chromosome 11 Genes vs. Genes from Other Genomic Locations. To place the above findings into perspective with previous reports of DNA hypomethylation in human tumors, we studied the methylation status of C+G-rich regions in DNA samples from 7 human tumors selected to demonstrate a full range of *CALC1* gene methylation patterns (Fig. 1*A*). Four genes on the short arm of chromosome 11, from the centromere toward the terminal portion, including catalase at 11p13 (33), *CALC1* at 11p15 (18, 19), *HRAS* at 11p15.5 (43, 44), and an anonymous region termed pADJ 762 at 11p15.5 (29) had a remarkable concordance for increased numbers of methylated CCGG sites (Fig. 1). Three of the 4 tumors that exhibited the greatest degree of *CALC1* gene methylation (Fig. 1*A*, lanes 4, 6, and 7) also had increased CCGG methylation in pADJ 762 (Fig. 1*B*, lanes 6, 8, and 9), *HRAS* (Fig. 1*C*, lanes 9, 11, and 12), and catalase (Fig. 1*D*, lanes 4, 6, and 7). In one cancer showing only a moderate increase in *CALC1* gene methylation (Fig. 1*A*, lane 8) two of the three other chromosome 11 regions were also moderately hypermethylated (Fig. 1*C*, lane 13; Fig. 1*D*, lane 8), and the other was normally methylated (Fig. 1*B*, lane 10). In DNA from two tumors hypomethylated for the *CALC1* gene (Fig. 1*A*, lanes 9 and 10), pADJ 762 and the catalase gene were also hypomethylated (Fig. 1*B*, lanes 11 and 12; Fig. 1*D*, lanes 9 and 10), while *HRAS* gene methylation was similar to normal tissues in one tumor (Fig. 1*C*, lane 14) and hypomethylated in the other (lane 15). Only one tumor DNA, extracted from a small cell lung carcinoma sample, was differentially methylated for the four chromosome 11 genes studied, exhibiting hypermethylation for *CALC1* (Fig. 1*A*, lane 5), normal methylation for pADJ 762 (Fig. 1*B*, lane 7), and hypomethylation for *HRAS* (Fig. 1*C*, lane 10) and catalase (Fig. 1*D*, lane 5).

In contrast to the above findings, genes examined on other chromosomes (three examples given; Fig. 2) had no increased CCGG methylation. In the four tumor DNA samples most extensively methylated for chromosome 11 genes, the *MYC* gene on chromosome 8 (46, 47) had no methylation of 5′ CCGG sites (Fig. 2*A*, lanes 5–10). Similarly, the methylation patterns of the *FOS* gene (Fig. 2*B*) on chromosome 14 (48)

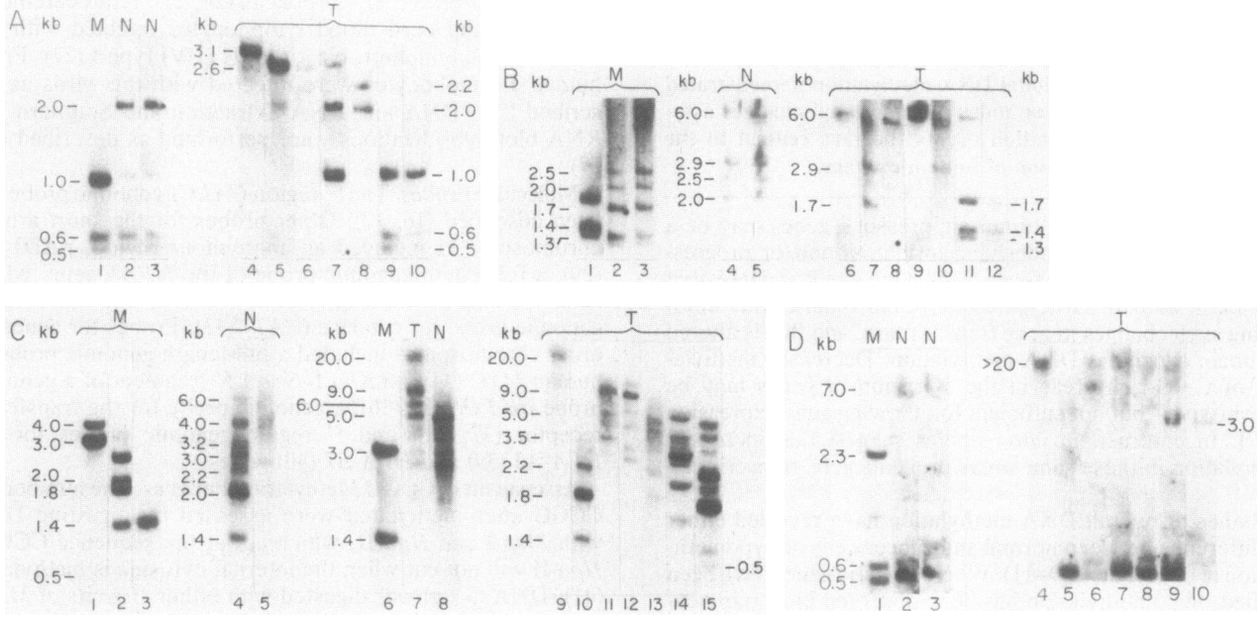

FIG. 1. Tumor-associated CCGG methylation in selected chromosome 11 regions. (*A*) Calcitonin. A representative *Msp* I pattern (example for lung) is shown in lane 1. Lanes 2 and 3 (lung and liver), *Hpa* II patterns typical for normal tissues. The decrease in the 1.0-kb band seen with *Msp* I and appearance of a 2.0-kb band are due to methylation of site M_5 (refs. 16 and 17; see also Fig. 4). Lanes 4–10, *Hpa* II pattern of 7 human tumor DNAs. Extensive methylation is present in teratocarcinoma (lane 4), small cell lung carcinoma (SCLC) (lanes 5 and 6), acute lymphocytic leukemia (lane 7), and modest hypermethylation in a non-SCLC lung cancer (lane 8). A rare pattern of hypomethylation is represented by medullary thyroid carcinoma (lane 9) and a culture of SCLC (lane 10). (*B*) pADJ 762. Lanes 1–3, *Msp* I digests; lanes 4 and 5, *Hpa* II digests typical for normal tissues. Lanes 6–12, the same *Hpa* II digests, respectively, shown in lanes 4–10 of *A*. Note extensive methylation (lanes 6, 8, and 9), normal methylation (lanes 7 and 10), and hypomethylation (lanes 11 and 12). (*C*) *HRAS*. The CCGG sites in the 3′ region of the *HRAS* gene are highly polymorphic (12, 13). We determined the full range of *Msp* I polymorphisms in normal and tumor tissues (lanes 1–3) and the range of *Hpa* II patterns in normal tissues (lanes 4, 5, and 8). We then compared *Hpa* II and *Msp* I patterns for each individual tumor. Extensive methylation was seen (lanes 9, 11, and 12), representing 3 of the 4 tumors hypermethylated for *CALC1* (lanes 4, 6, and 7 of *A*). The fourth tumor was hypomethylated for *HRAS* (lane 10). To prove further that these differences were not simply due to polymorphisms, we studied tumor and corresponding normal DNA from the same patient (lanes 6–8). The *Msp* I pattern was identical in both normal and tumor samples (lane 6). The *Hpa* II digest of leukemia blast cells (lane 7) showed extensive methylation compared to the normal blood cells after disease remission (lane 8). Identical results were previously seen in these same samples for the *CALC1* gene (17). (*D*) Catalase. Lane 1, *Msp* I digest typical of normal and tumor DNA; lanes 2 and 3, *Hpa* II digests typical of normal tissues. Lanes 4–10, the same *Hpa* II digests of tumor DNA shown in *A*, *B*, and *C*. DNA methylation patterns in lanes 4, 6, 7, and 8 are concordant with the increased methylation of the *CALC1* gene (lanes 4, 6, 7, and 8 in *A*). Hypomethylation is seen in lanes 9 and 10, as was true for the *CALC1* gene (lanes 9 and 10 in *A*). The same tumor, which deviated from the *CALC1* gene pattern for pADJ 762 and *HRAS*, also was less methylated for catalase (lane 5). M, *Msp* I; N, normal; and T, tumor cut with *Hpa* II.

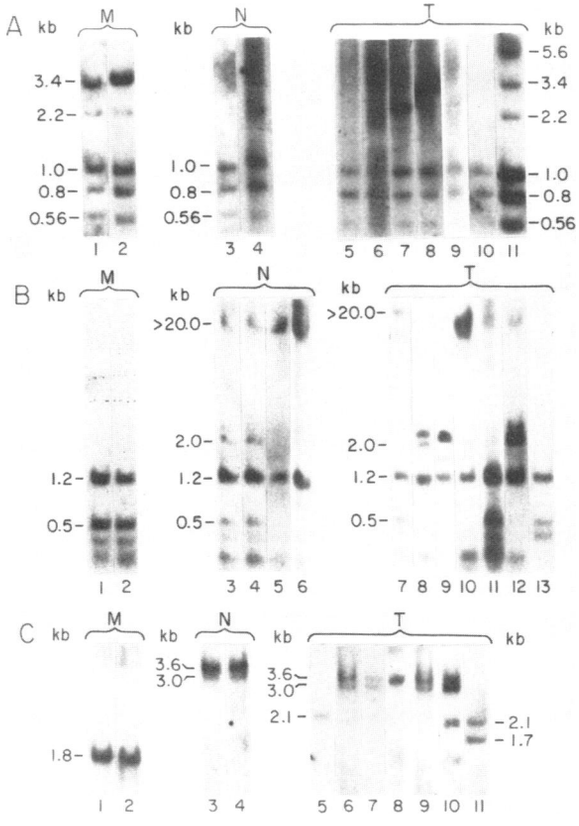

FIG. 2. Tumor-associated CCGG methylation in selected non-chromosome 11 genes. (*A*) *MYC*. Lanes 1 and 2, typical *Msp* I digests of normal DNA (lung and liver). C+G-rich areas of the 5′ flanking region and first exon account for the bands of 1.0, 0.8, and 0.56 kb, while infrequent CCGG sites in the 3′ region of the gene result in the 3.4- and 2.2-kb bands (45). In normal tissues (lanes 3 and 4), the 5′ sites are unmethylated, while the 3′ sites are methylated with resultant loss of the 3.4- and 2.2-kb bands. In the tumors (lanes 5–11) representing the same DNA samples, in order, as shown in lanes 4–10 of Fig. 1*A*, *Hpa* II digests are either identical to normal DNA (lanes 5, 6, 8, 9, and 10) or show relative hypomethylation (lanes 7 and 11) of the 3′ CCGG sites. The SCLC line NCI-H82 (lane 11) contains an amplified *MYC* gene (45). (*B*) *FOS*. Normal tissues show several methylated CCGG sites (compare *Hpa* II digests in lanes 3–6 to *Msp* I digests in lanes 1 and 2). In the tumor DNA (lanes 7–13), *Hpa* II patterns were either similar to normal (lanes 7, 10, and 11) or revealed relative hypomethylation (lanes 8, 9, 12, and 13). (*C*) Transferrin receptor. Normal tissues are considerably methylated at CCGG sites (*Hpa* II patterns in lanes 3 and 4 compared to *Msp* I patterns in lanes 1 and 2). The seven tumor DNA samples (lanes 5–11) are either methylated identically to normal tissues (lanes 6, 7, 8, and 9) or are relatively hypomethylated (lanes 5, 10, and 11).

and the transferrin receptor gene (Fig. 2*C*) on chromosome 3 (49) were either identical to normal tissues (Fig. 2*B*, lanes 7, 10, and 11; Fig. 2*C*, lanes 6, 7, 8, and 9) or hypomethylated (Fig. 2*B*, lanes 8, 9, 12, and 13; Fig. 2*C*, lane 5, 10, and 11). Interestingly, both tumors hypomethylated for *CALC1* (Fig. 1*A*, lanes 9 and 10) were frequently hypomethylated in each of the genes on other chromosomes (Fig. 2*A*, lane 11; Fig. 2*B*, lanes 12 and 13; Fig. 2*C*, lanes 10 and 11). Finally, the *HLA-A2* and *HLA-B7* genes on chromosome 6 (39, 40) were also either normally methylated or hypomethylated in all the same tumors studied (data not shown).

Changes in *CALC1* Gene Methylation Induced by Transforming Viruses. The high frequency of DNA methylation changes in human cancers makes it important to time their appearance during the transformation process and define the causative steps. To determine whether the hypermethylation changes on chromosome 11 are also an early neoplastic event, we investigated the methylation status of the *CALC1* gene during viral transformation of human cells.

Since culture alone can induce changes in DNA methylation (24, 25), we first examined uninfected young and senescing fibroblasts. Cultured fibroblasts actually develop, with time, as previously reported for genomic DNA methylation (24, 25), a tendency to hypomethylation of the *CALC1* gene (Fig. 3, lanes 1 and 2). By contrast, simian virus 40-infected cells develop aberrant methylation at some of the same CCGG sites involved in human tumors (lane 3).

More directly applicable to human cancers are findings with T-cell lymphotropic viruses (HTLVs). Normal T cells in culture induced to proliferate by interleukin 2 develop reduced methylation of the *CALC1* gene (Fig. 3, lane 5 compared to lane 4). In contrast, HTLV induces the new sites of CCGG methylation previously seen in leukemias (17). The CT gene is extensively methylated in a HTLV-I-positive T-cell lymphoma (lane 6), in long-term cultures of HTLV-I-positive T-cell leukemia (lane 7), and HTLV II-associated leukemia (lane 8). Established lines of adult human T cells (lane 9) and normal cord blood T cells (lane 10) infected *in vitro* with HTLV-I also show extensive hypermethylation. Most importantly, acutely infected T cells become abnormally methylated within 8 weeks of exposure to HTLV-I (lane 11), demonstrating that abnormal DNA methylation can occur relatively early during the transformation process, before the evolution of frank tumorigenicity.

Correlation of *CALC1* Gene Methylation with Conformation of the Transcription Regulatory Region. DNA hypermethylation in cancer cells could have important functional implications. Recent studies suggest that demethylation of gene regulatory areas may not alone alter gene transcription (6, 7). In contrast, *in vitro* studies have shown that extensive cytosine methylation within C+G-rich "islands" in the 5′ regions of genes may result in a marked reduction of transcriptional activity (8). DNA hypermethylation could then provide one mechanism for the loss of gene function thought to underlie initiation and progression of human cancers (1–5). Consequently, we have examined the chromatin structure of the *CALC1* gene in cells that exhibit various degrees of *CALC1* gene methylation and expression.

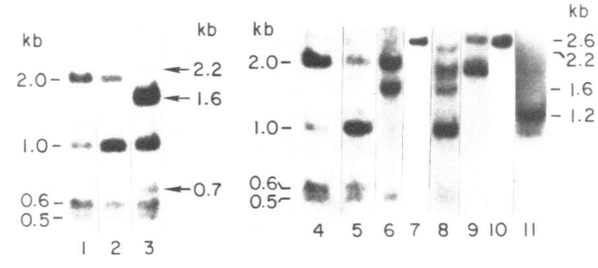

FIG. 3. *CALC1* gene *Hpa* II digestion patterns in cells infected with transforming viruses. Human fibroblasts (lane 1), passage 20, had a normal pattern of methylation involving site M_5 (see Fig. 4 *Upper*) but showed decreased methylation (lane 2) of this site during senescence (passage 50; note decrease in the 2.0-kb band and increase in the 1.0-kb band). Simian virus 40 infection of human fibroblasts produced new methylation patterns (lane 3) for 5′ sites (note loss of the normal 2.0-kb band and appearance of abnormal bands at 2.2, 1.6, and 0.7 kb). Normal T lymphocytes stimulated to grow with interleukin 2 (obtained from Alan Hess, Johns Hopkins Oncology Center) undergo decreased methylation at site M_5 (lanes 4 and 5). T cells infected with HTLV retroviruses become hypermethylated. Note the abnormal 1.6-, 2.2-, and/or 2.6-kb bands in DNA from a HTLV-I-positive T lymphoma (lane 6), from established lines of HTLV-I-positive T-cell leukemia (lane 7), and HTLV-II leukemia (lane 8). Cells infected with HTLV-I by coculture also had extensive methylation as seen in established lines of adult T cells (lane 9), umbilical cord blood T cells, MT2 line (27) (lane 10), as well as within 2 months after infection of adult T cells (lane 11).

Our studies focused on a region located from −1000 to +200 base pairs relative to the start of exon 1, which is important for the induction of transcription of the *CALC1* gene *in vitro* (refs. 50 and 51; B.D.N. and A.d.B., unpublished data).

The TT line of human medullary thyroid carcinoma (MTC) (50–52) and the DMS 53 (53) line of human small cell lung carcinoma (SCLC) highly transcribe the *CALC1* gene (data not shown) and are hypomethylated at CCGG sites in the *CALC1* gene (example for MTC, Fig. 1*A*, lane 9). Both cell types exhibited (Fig. 4) the fully open chromatin pattern typical for transcriptionally active genes. *Msp* I sites M_2, M_3, and M_4 (see Fig. 4 *Upper*) are fully accessible in whole nuclei to cleavage with this enzyme and sub-bands are generated within a 4.3-kilobase (kb) *Bgl* II and 2.7- and 1.9-kb *Bam*HI fragments as shown (Fig. 4 *Lower*). Other enzyme sites within this region (Fig. 4 *Upper*) such as *Ban* II, *Bam*HI, *Ava* I, and *Bgl* II were also accessible (data not shown).

One line of SCLC, NCI H82 (22), and a line of normal fibroblasts exhibit 5′ region hypomethylation but do not transcribe the *CALC1* gene (data not shown). Both cell types had a "partially" open *CALC1* gene conformation (see H82 in Fig. 4 *Lower*). Thus, sites M_3 and M_4 are only partially accessible to digestion and site M_2 does not cleave. These results fit the hypothesis that hypomethylation in the 5′ region does not, directly or alone, fully dictate the conformation or expression level of genes and that other cellular factors must be operative (6–8).

Two cell lines, the OH3 line (21) of SCLC and the Colo 320 line of colon carcinoma (34), have extensive methylation of (patterns such as those in Fig. 1*A*, lanes 5–7) and do not transcribe the *CALC1* gene (data not shown). In these cells, the *CALC1* gene appears to be in a fully "closed" conformation. No. 5′ *Msp* I sites were accessible to digestion (see OH3 in Fig. 4 *Lower*). This closed conformation is specific for *CALC1*, since the *MYC* gene, which is unmethylated in its 5′ region in these cell lines (examples in Fig. 2, lane 7), showed equal accessibility of *Msp* I sites in known DNase I hypersensitive regions (45) (data not shown).

The Tera-I line of teratocarcinoma cells (26), which are hypermethylated for (Fig. 1*A*, lane 4) and do not spontaneously express the *CALC1* gene (data not shown), showed, surprisingly, a fully open conformation pattern (Fig. 4). Teratocarcinomas, unlike lung and colon cancers, are neoplasms of embryonic rather than mature somatic cells, and

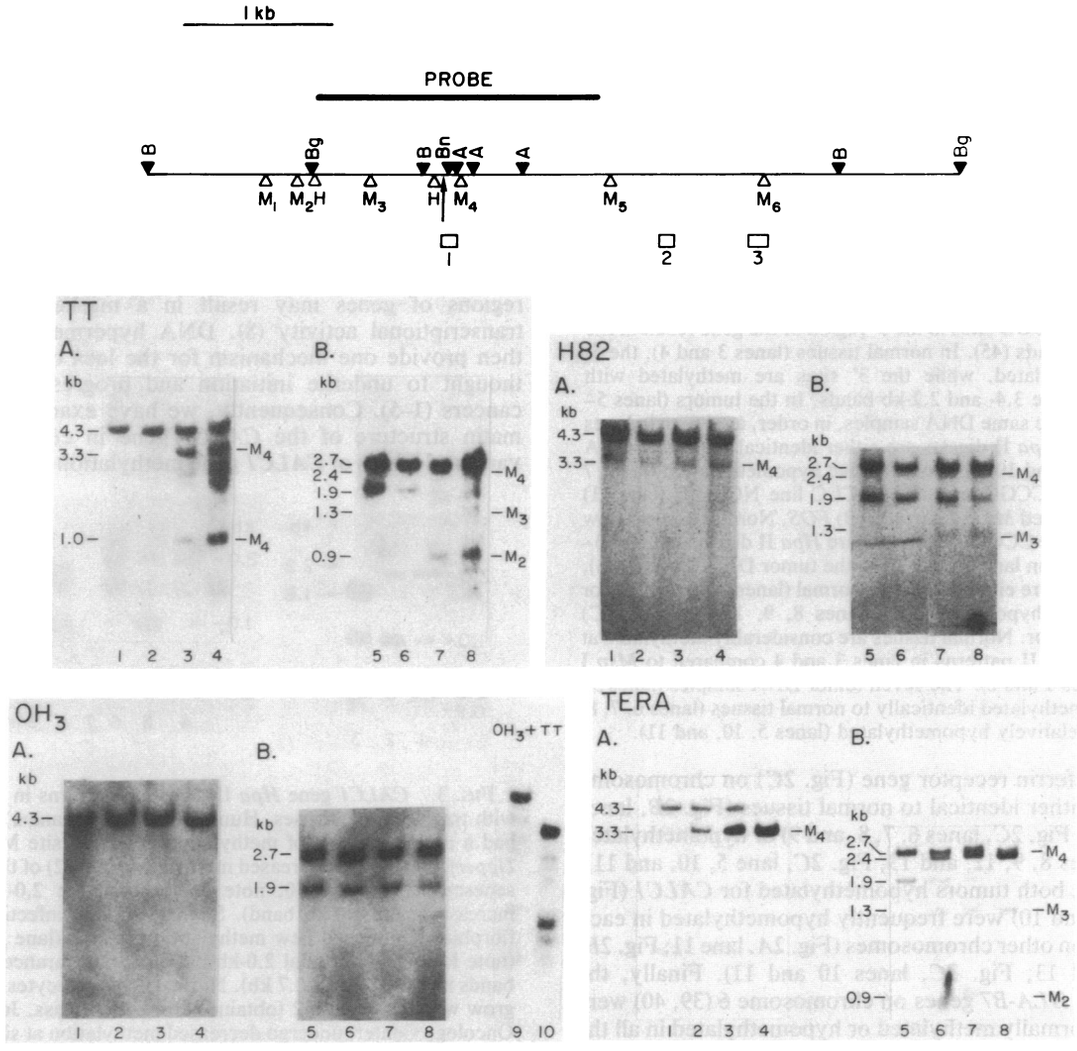

FIG. 4. (*Upper*) Abbreviated map of the 5′ region of the *CALC1* gene showing the first 3 of 6 exons (squares) and restriction sites of interest as well as the position of the 1.7-kb genomic probe (16, 17). M, *Msp* I; H, *Hha* I; A, *Ava* I; Bg, *Bgl* II; B, *Bam*HI; Bn, *Ban* II. (*Lower*) Chromatin structure of the 5′ end of the *CALC1* gene in human cancer cell types with different degrees of *CALC1* gene methylation. *Msp* I was incubated with nuclei from each cell line as described and was used predominantly because of the clustering of CCGG sites at the 5′ end of the *CALC1* gene. The sub-bands generated for *Msp* I sites accessible to cleavage are shown at the right of each panel. OH3, lanes 9 and 10 represent *Bgl* II and *Bam*HI digests of a 1:1 mixture of OH3 and TT nuclei, digested with 450 units of *Msp* I per ml. The 50% digestion pattern for this mixture helps rule out the presence of a restriction enzyme inhibitor in OH3 nuclei.

Groudine and Conkin (54) have shown that extensive 5′ region methylation and nuclease hypersensitivity may transiently coexist during early embryonic development.

DISCUSSION

We have now shown that the abnormalities of DNA methylation frequently found in human cancer include not only widespread hypomethylation but also regional hypermethylation on chromosome 11. Furthermore, this increased methylation occurs early in viral transformation of human cells and is associated, in common somatic cell tumor types, with a closed transcriptional site conformation in the *CALC1* gene. This latter finding stresses the potential relationship between hypermethylation and gene inactivation. Precedent for association between altered chromosome structure, increased methylation, and gene inactivation is also found in normal cells. During, or shortly after, inactivation of one X chromosome in female mammals, sites unmethylated in the active X chromosome become methylated in genes no longer transcriptionally active (55, 56).

In transformed cells, aberrant increases in DNA methylation could thus have similar implications for chromosomal structure and gene expression. The region in which we have demonstrated a hot spot for increased DNA methylation in human cancer is particularly important. The short arm of chromosome 11 is morphologically and/or functionally altered in several human cancers (3–5). This chromosome also harbors genes whose normal expression may be integral to preventing emergence of the cancer phenotype in some cell systems (3–5). Structural abnormalities that might inactivate key regions of chromosome 11, either marked or caused by increased DNA methylation, could thus be an important step in the formation or progression of important types of human tumors.

We thank Drs. Adi Gazdar, Joel Schindler, Samuel Goldstein, Olive Pettengill, and George Sorenson for provision of human tumor cell lines; Dr. Robert Korneluk for the catalase gene probe (12); Dr. Robert Casero for critical review of the manuscript; Ms. Tammy Hess and Sande Lund for secretarial assistance; and Ms. Kathy Wieman for technical assistance and figure preparation. Portions of this work were supported by Grant 1 RO1 CA 43318-01 from the National Cancer Institute, a grant from the Tobacco Research Foundation, a gift from the Clayton Fund, and a Veterans' Administration Clinical Investigation Grant to B.P. A.d.B. is supported, in part, by the Hodson Trust Foundation.

1. Francke, U. (1983) in *Chromosomes and Cancer*, eds. Rowley, J. D. & Ultmann, J. E. (Academic, New York), Vol. 5, pp. 99–116.
2. Knudson, A. G. (1986) *Annu. Rev. Genet.* **20,** 231–251.
3. Scrable, H. J., Lampkin, B. C., Witte, D. P. & Cavenee, W. K. (1987) *Nature (London)* **329,** 645–647.
4. Saxon, P. J., Srivatsan, E. S. & Stanbridge, E. J. (1986) *EMBO J.* **5,** 3461–3466.
5. Weissman, B. E., Saxon, P. J., Pasquale, S. R., Jones, G. R., Geiser, A. G. & Stanbridge, E. J. (1987) *Science* **236,** 175–180.
6. Bird, A. P. (1986) *Nature (London)* **321,** 209–213.
7. Holliday, R. (1987) *Science* **238,** 163–169.
8. Keshet, I., Lieman-Hurwitz, J. & Cedar, H. (1986) *Cell* **44,** 535–543.
9. Gama-Sosa, M. A., Slagel, V. A., Trewyn, R. W., Oxenhandler, R., Kuo, K., Gehrke, C. W. & Ehrlich, M. (1983) *Nucleic Acids Res.* **11,** 6883–6894.
10. Jones, P. A. (1986) *Cancer Res.* **46,** 461–466.
11. Flatau, E., Bogenmann, E. & Jones, P. A. (1983) *Cancer Res.* **43,** 4901–4905.
12. Feinberg, A. P. & Vogelstein, B. (1983) *Nature (London)* **301,** 89–92.
13. Feinberg, A. P. & Vogelstein, B. (1983) *Biochem. Biophys. Res. Commun.* **111,** 47–54.
14. Goelz, S. E., Vogelstein, B., Hamilton, S. R. & Feinberg, A. P. (1985) *Science* **228,** 187–190.
15. Kautainien, T. L. & Jones, P. A. (1986) *J. Biol. Chem.* **261,** 1594–1598.
16. Baylin, S. B., Hoppener, J. W. M., de Bustros, A., Steenbergh, P. H., Lips, C. J. M. & Nelkin, B. D. (1986) *Cancer Res.* **46,** 2917–2922.
17. 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.
18. Przepiorka, D., Baylin, S. B., McBride, O. W., Testa, J. R., de Bustros, A. & Nelkin, B. D. (1984) *Biochem. Biophys. Res. Commun.* **120,** 493–499.
19. Hoppener, J. W. M., Steenbergh, P. H., Zandberg, J., Bakker, E., Pearson, P. L., Geurts van Kessel, A. H. M., Jansz, H. S. & Lips, C. J. M. (1984) *Human Genet.* **66,** 309–312.
20. Stenman, G. & Sager, R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9099–9102.
21. Griffin, C. A. & Baylin, S. B. (1985) *Cancer Res.* **45,** 272–275.
22. Gazdar, A. F., Carney, D. N., Nau, M. M. & Minna, J. D. (1985) *Cancer Res.* **45,** 2924–2930.
23. Park, J. G., Kramer, B. S., Steinberg, S. M., Carmichael, J., Collins, J. M., Minna, J. D. & Gazdar, A. F. (1987) *Cancer Res.* **45,** 5875–5879.
24. Schmookler Reis, R. J. & Goldstein, S. (1982) *Nucleic Acids Res.* **10,** 4293–4304.
25. Schmookler Reis, R. J. & Goldstein, S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3949–3953.
26. Fogh, J. (1978) *Natl. Cancer Inst. Monogr.* **49,** 5–19.
27. Miyoshi, I. (1981) *Nature (London)* **294,** 770–771.
28. Graziano, S. L., Lehr, B. M., Merl, S. A., Ehrlich, G. D., Moore, J. L., Hallinan, E. J., Hubbell, C., Davey, F. R., Vournakis, J. & Poiesz, B. J. (1987) *Cancer Res.* **47,** 2468–2473.
29. White, R., Leppert, M., Bishop, T. D., Barker, D., Berkowitz, J., Brown, C., Callahan, P., Holm, T. & Jerominski, L. (1985) *Nature (London)* **313,** 101–105.
30. Shih, C. & Weinberg, R. A. (1982) *Cell* **29,** 161–169.
31. Tabin, C. J., Bradley, S. M., Bargmann, C. I., Weinberg, R. A., Papageorge, A. G., Scolnick, E. M., Dhar, R., Lowy, D. R. & Chang, E. H. (1982) *Nature (London)* **300,** 143–152.
32. Reddy, E. P. (1983) *Science* **220,** 1061–1063.
33. Quan, F., Korneluk, R. G., Tropak, M. B. & Gravel, R. A. (1986) *Nucleic Acids Res.* **14,** 5321–5335.
34. Alitalo, K., Schwab, M., Linn, C. C., Varmus, H. E. & Bishop, J. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1707–1711.
35. Curran, T. (1983) *Mol. Cell. Biol.* **3,** 914–921.
36. Miller, A. D., Curran, T. & Verma, I. M. (1984) *Cell* **36,** 51–60.
37. Kuhn, L. C., McClelland, A. & Ruddle, F. H. (1984) *Cell* 37, 95–103.
38. McClelland, A., Kuhn, L. C. & Ruddle, F. H. (1984) *Cell* **39,** 267–274.
39. Barbosa, J. A., Kamarck, M. E., Biro, P. A., Weissman, S. M. & Ruddle, F. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6327–6331.
40. Sood, A. K., Pereira, D. & Weissman, S. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 616–620.
41. Van der Ploeg, L. H. T. & Flavell, R. A. (1980) *Cell* **19,** 947–958.
42. Sweet, R. W., Chao, M. V. & Axel, R. (1982) *Cell* **31,** 347–353.
43. McBride, O. W., Swan, D. C., Santos, E., Barbacid, M., Tronick, S. R. & Aaronson, S. A. (1982) *Nature (London)* **300,** 773–774.
44. Jhanwar, S. C., Neel, B. G., Hayward, W. S. & Chaganti, R. S. K. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4794–4797.
45. Seifter, E. J., Sausville, E. A. & Battey, J. (1986) *Cancer Res.* **46,** 2050–2055.
46. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7824–7827.
47. Neel, B. G., Jhanwar, S. C., Chaganti, R. S. K. & Hayward, W. S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7842–7846.
48. Barker, P. E., Rabin, M., Watson, M., Breg, W. R., Ruddle, F. H. & Verma, I. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5826–5830.
49. Miller, Y. E., Jones, C. & Scroggin, C. (1983) *Am. J. Hum. Genet.* **35,** 573–583.
50. de Bustros, A., Baylin, S. B., Berger, C. L., Roos, B. A., Leong, S. S. & Nelkin, B. (1985) *J. Biol. Chem.* **260,** 98–104.
51. de Bustros, A., Baylin, S. B., Levine, M. A. & Nelkin, B. D. (1986) *J. Biol. Chem.* **261,** 8036–8041.
52. Leong, S. S., Horoszewicz, J. S., Shimaoka, K., Friedman, M., Kawinski, E., Song, M. J., Zeigel, R., Chu, T. M., Baylin, S. & Mirand, E. A. (1981) in *Advances in Thyroid Neoplasia*, eds. Andreoli, M., Monaco, F. & Robbins, J. (Field Educational Italia, Rome, Italy), p. 95.
53. Edbrooke, M. R., Parker, D., McVey, J. H., Riley, J. H., Sorenson, G. D., Pettengill, O. S. & Craig, R. K. (1985) *EMBO J.* **4,** 715–724.
54. Groudine, M. & Conkin, K. F. (1985) *Science* **228,** 1061–1068.
55. Wolf, S. F., Jolly, D. J., Lunnen, K. D., Friedmann, T. & Migeon, B. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2806–2810.
56. Lock, L. F., Takagi, N. & Martin, G. R. (1987) *Cell* **48,** 39–46.