Different levels of DNA modification at 5'CCGG in murine erythroleukemia cells and the tissues of normal mouse spleen

Steven S. Smith^{*}, John C. Yu[†] and Carton W. Chen^{*†}

Departments of ^{*}Cellular Biology and [†]Immunopathology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

Received 12 April 1982; Accepted 21 June 1982

ABSTRACT

The DNA of a Friend erythroleukemic cell line (clone DS-19) and that of mouse spleen from which it was derived were compared with respect to modification at 5'CCGG. Methylation patterns in clone DS-19 DNA were very different from those in normal spleen DNA. Our results suggest that the mechanism by which the internal cytosine in the 5'CCGG sequence is modified has been partially inactivated in clone DS-19. We observed a general decrease of about two-fold in total modification at this site in DS-19 DNA. This general decrease was shown to extend to 5'CCGG sites in specific classes of repeated sequences with two-dimensional displays of restriction fragments. Interspersed repeated sequences which are concertedly modified (*i.e.*, modified at many different chromosomal locations) in spleen, were found to be unmodified at these locations in the cell line. However, we did not detect a difference in DNA modification at 5'CCGG with these techniques when uninduced and hexamethylene-bis-acetamide (HMBA) induced cells of clone DS-19 were compared. In other experiments, we obtained further evidence that selected classes of repeated sequences in Physarum polycephalum are methylated to the same extent independent of chromosomal location.

INTRODUCTION

The function of the postsynthetic methylation of DNA in eukaryotes is not understood. In the vertebrates, 5-methyl-cytosine (M) is the only modified base thus far detected,¹ where it occurs primarily in the palindromic sequence 5'CG.^{2,3} The presence of two-fold rotational symmetry in this sequence led Holliday and Pugh⁴ and Riggs⁵ to suggest a model for the somatic inheritance of DNA methylation patterns in which a "maintenance" methylase would recognize the hemi-methylated sequence after DNA replication, thus reproducing the pattern of modification in both daughter strands. They went on to suggest that modification of these sequences might be a central part of the molecular mechanisms of X-inactivation and the control of gene expression at the level of cell commitment. Since that time, the use of the restriction enzymes HpaII and MspI⁶ in studies of DNA modification has provided support for both a possible role in the potentiation of gene expression, and the existence of

mechanisms for the accurate somatic inheritance of patterns of DNA modification. (For recent reviews, see references 7, 8, and 9.)

In view of the possibility that DNA modification may play a role in at least the potentiation of gene expression, we have investigated the modification of the DNA of the Friend cell line both before and after induction with (HMBA). In this study we have used both one-dimensional and two-dimensional displays of restriction fragments to search for changes in modification at 5'CCGG. No differences in modification state were observed with these techniques as a function of induction. However, a comparison of the one-dimensional and two-dimensional displays of the Friend cell DNA with that of normal spleen tissue from Balb/c and DBA/J2 mice shows that the Friend cell line maintains its DNA in a lower modification state at 5'CCGG than that of the normal spleen tissue from which the cell line was originally derived.¹⁰

MATERIALS AND METHODS

DNA Preparations

DBA/J2 mouse spleen DNA was prepared by the method of Blin and Stafford¹² except that a mortar and pestal was used to grind the spleens to a frozen powder under liquid nitrogen.

Balb/c mouse spleen DNA (the gift of Dr. J. Garrett Reilly) was prepared by the method of Mantueil *et al.*¹¹ except that proteinase K digestion (100 µg/ml at 55°C for 15 hrs) was substituted for the pronase digestion step employed by these authors.

Subclone DS-19 of Friend erythroleukemic cell line 745 derived from DBA/J2 mouse spleen^{10,13} was cultured as previously described.¹⁴ After induction of globin with 4 mM HMBA was carried out,¹⁴ the percentage of hemoglobin positive cells was more than 95% as judged by the benzidine staining assay.^{15,16}

DS-19 DNA was isolated from nuclei purified by sedimentation through 0.25 M sucrose onto a 0.88 M sucrose cushion after lysis in 20 packed cell volumes of 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 1.5 mM MgCl₂. NP-40 detergent was used to lyse the cells. The concentration of NP-40 necessary to obtain 100% cell lysis (usually between 0.1% and 0.5%) was determined by phase contrast microscopy. Isolated nuclei were then lysed in 10 mM Tris-HCl, pH 8, 10 mM ethylenediamine tetraacetic acid 10 mM NaCl and 0.5% sodium dodecyl sulfate. Proteinase K was added to 100 µg/ml and the viscous mixture was warmed at 37°C for 30 min. Afterwards, an additional 50 µg/ml Proteinase K was added and the mixture was incubated for another 60 min at 65°C. The solution was then

phenol extracted, chloroform extracted, ether extracted and dialyzed against 10 mM Tris, 1 mM ethylenediamine tetraacetic acid, pH 7.5.

Physarum polycephalum DNA was prepared from M₃C macroplasmoidal surface cultures harvested in G2 phase of the cell cycle. Nuclei were isolated by the method of Mohberg and Rusch¹⁷ and DNA was prepared essentially as described by Hall, Turnock and Cox.¹⁸

The molecular length of all DNA preparations above was judged to be greater than 50 kb by comparison with full length λ phage DNA on 0.5% agarose gels.

Restriction Enzyme Digestion and End-labeling

Restriction enzyme digestion conditions and the controls employed to test for the completeness of digestion have been previously described.¹⁹ The methods used for end-labeling of restriction fragments by terminal repair were also as previously described¹⁹ except that α -³²P-dGTP at 800 Ci/mmol, in Tricine buffer (New England Nuclear) was used.

Preparation of Two-Dimensional Displays

Methods used in the preparation of two-dimensional displays were also as described previously¹⁹ except that the first dimension separation gel was 1.1% "LEEO" agarose. The separation gel itself was shortened to 12 cm and the DNA was loaded in a slot 10 cm from the end of the gel. First dimension electrophoresis was carried out with a voltage gradient of 3 V/cm measured in the gel. The pumping rate was 13.2 ml/hr throughout the separation. Fifty-six fractions were collected at 23 min intervals.

Cleavage Frequency Determinations

We employed the method of Botchan²⁰ et al. to determine cleavage site frequencies. In these experiments DNA digested with HpaII, or MspI was separated on one dimensional agarose gels. After electrophoresis, the gels were stained in water containing 0.5 μ g/ml ethidium bromide for 1 hr at room temperature. The gels were then photographed with Polaroid-type 55 film. Scans of the photographic negatives were prepared with the aid of a Kontes Model 800 Fiber Optic Scanner (Kontes Scientific Instrument Group, Vineland, NJ). Several photographs of each gel were taken at different exposure times. Negatives were selected for further analysis when the ratio of scanned areas in the lanes containing λ DNA fragments were approximately equal to the ratio of their known molecular weights. The lanes containing mouse DNA digests were then scanned and used to determine the restriction site cleavage frequencies as follows. The weight fraction (F(L)) of the DNA between length zero and the length L for each of four markers in each scan was determined from the ratio

of the area of the scan from the bottom of the gel to L, to the total area of the scan. This fraction is given by the expression of Botchan *et al.*²⁰:

$$F(L) = \int_0^L L^2 f e^{-fL} = 1 - (1+fL)e^{-fL},$$

where f is the frequency of the restriction site cleaved by a given enzyme. Standard curves of $(1+fL)e^{-fL}$ as a function of f were prepared for each value of L used in the analysis. Since this function is equal to $1-F(L)$ one can use these curves to determine values of f at each molecular weight marker in the scan. The values given in Table 1 are the results of two separate determinations at several values of L per determination. For further details on the use of this method and its validity see refs. 20 and 21.

RESULTS

One-dimensional Displays

Singer, Roberts-Ems, and Riggs²² have shown that mouse liver DNA is highly modified at the 5'CCGG sequence recognized by HpaII and MspI. We employed a similar technique to obtain information on the level of modification at this site in the Friend erythroleukemic cell line and the tissues of spleen from which this cell line was derived.¹⁰ In these experiments high molecular weight DNA was isolated and digested to completion with either HpaII or MspI. Samples of the digestion product containing 1.5 μ g each were then separated by electrophoresis in agarose gels of various percentages in 50 mM Tris, 10 mM ETDA, 30 mM sodium acetate, pH 7.8. After electrophoresis, the gels were stained with ethidium bromide and photographed. A representative photograph is shown in Figure 1. In this figure, DNA from DBA/J2 spleen cells is compared with DNA from Friend erythroleukemic cells, and DNA from these cells isolated after treatment with HMBA. No significant difference in cleavage pattern was observed in Friend cell DNA as a function of HMBA treatment, but a clear difference was observed when digests of normal spleen DNA were compared with those of DNA from the Friend cell line.

A qualitative comparison of each digest in Figure 1 shows that DBA/J2 mouse spleen DNA and DS-19 DNA are digested more extensively by MspI than by HpaII as expected from the work of others.²² However, DNA from the Friend cell line is digested to a greater extent by HpaII than normal spleen DNA. The simplest conclusion one can draw from this is that the DNA of the Friend cell line is much less extensively modified at 5'CCGG than that of the spleen tissue from which it was derived.

In order to quantitate the data, cleavage frequencies for HpaII (f_{Hpa}) and MspI (f_{Msp}) were determined from scans of photographs like that shown in

Figure 1. The Extent of DNA Modification at 5'CCGG Using Restriction Digests of Total Mouse DNA. High molecular weight DNA was isolated from DBA/J2 mouse spleens, Friend cells (clone DS-19), and Friend cells induced with hexamethylene-bis-acetamide. Samples of each type of DNA were digested to completion with HpaII or MspI. After digestion the fragments were separated on agarose gels of various percentages. A representative result with 1.0% agarose is shown in the figure. Lane 1, DBA/J2 (HpaII digest); lane 2, DS-19 (HpaII digest); lane 3, HMBA induced DS-19 (HpaII digest); lane 4, positions of molecular length markers (kb); lane 5, DBA/J2 (MspI digest); lane 6, DS-19 (MspI digest); lane 7, HMBA induced DS-19 (MspI digest).

Figure 1, as described in Materials and Methods. The results of this analysis are given in Table 1, where it is seen that f_{Hpa} is about two-fold higher in DS-19 DNA than in DBA/J2 DNA, while f_{Msp} is about the same for both types of DNA. As noted by McClelland²³, the cleavage specificities of HpaII and MspI are complex. However, differences in HpaII cleavage frequencies are indicative of differences in the extent of methylation at the internal cytosine in the 5'CCGG sequence. Thus the simplest interpretation of the results is that methylation at this cytosine in 5'CCGG is about two times less frequent in DS-19 DNA than it is in DBA/J2 DNA.

TABLE 1. HpaII and MspI Cleavage Frequencies

DNA source	$f_{\text{Hpa}} \text{ (bp}^{-1}\text{)}$	$f_{\text{Msp}} \text{ (bp}^{-1}\text{)}$
DBA/J2 Spleen	$(0.7 \pm 0.1) \times 10^{-4}$	$(3.3 \pm 0.5) \times 10^{-4}$
DS-19*	$(1.3 \pm 0.1) \times 10^{-4}$	$(3.1 \pm 0.6) \times 10^{-4}$

*Average of data obtained with DNA from untreated and HMBA treated cells.

Two-Dimensional Displays

In view of the utility of the sequential digestion technique for the two-dimensional display of restriction fragments²⁴⁻²⁶ in obtaining information on the modification of eukaryotic repeated sequences^{19,27} we studied the modification of DNA from spleen and the Friend cell line further with this technique. The experiment shown in Figure 2 and the schematic drawing shown in Figure 3 illustrates the type of information which can be obtained. For the purpose of illustration, DNA from the lower eukaryote Physarum polycephalum was employed. The Physarum plasmodium has three advantages in studies of this type: first, it is a syncytium and therefore a single cell type; second, it possesses a high level of DNA modification²⁸⁻³¹; and third, it has a small genome.³²

In this experiment, high molecular weight DNA was digested to completion with BamHI, end-labeled, and fractionated by electroelution through agarose gel.¹⁹ The resulting fractions were then concentrated by alcohol precipitation, and identical samples of each fraction were digested to completion with HpaII or MspI. The digests were loaded into adjacent lanes of separate slab gels, with HpaII digests on the left and MspI digests on the right of each pair. After drying and autoradiography, a two-dimensional array was formed as a composite of the autoradiographs.

The combination of end-labeling, sequential digestion and the use of the HpaII/MspI isoschizomeric pair allows one to obtain several types of information. One type of information derives from the use of end-labeling in the procedure. This permits the use of the autoradiographic intensity of bands in the displays as a relative measure of the frequency of a class of sequence. In genomes as complex as those of Physarum (2.5×10^8 bp/haploid nucleus)³² and mouse (3.0×10^9 bp/haploid nucleus)³³ one expects to observe repeated sequences as intense bands above a continuum of unresolved DNA

Figure 2. Concerted Modification in the Repeated Sequences of *Physarum polycephalum*. High molecular weight DNA from microplasmodia of *Physarum polycephalum* was digested to completion with *Bam*HI, end-labeled and fractionated by electroelution. Identical samples of each fraction were digested to completion with either *Hpa*II or *Msp*I. The digests were loaded into adjacent lanes of four separate slab gels with *Hpa*II digests on the left and *Msp*I digests on the right in each pair to form an interdigitated array. Markers in the central lane of each panel indicate the position of DNA fragments produced by digesting λ DNA with *Bam*HI and *Eco*RI. Repeated sequences which form horizontal lines (marked C in the Figure) occupy different genomic locations. In this organism three prominent horizontal lines of this type are observed, and in each case, each repeat is completely modified at the 5'CCGG sequence inspected, independent of genomic location as explained in the schematic (Figure 3). Examples of repeated sequences which are not heavily modified are marked A. Examples of bands present only in the *Hpa*II lane are marked B.

Figure 3. Schematic of the 2-D Test for Concerted Modification. Cleavage of the DNA containing interspersed repeated sequences (darkened boxes) followed by end-labeling and fractionation by molecular weight are indicated by the arrow marked 1. An arrow marked 2 denotes a 5'CCGG recognition sequence in each repeat. The fragments are shown in order of increasing molecular weight from left to right. The symbol (*) denotes labeled ends. In the processes marked 2, samples of each fraction are digested with either *HpaII* or *MspI*, and the samples are separated in adjacent lanes of the slab gel with *HpaII* digests (H) on the left and *MspI* digests (M) on the right. Darkened bars in panels A and B represent autoradiographic bands of identical mobility which arise from the repeated sequences form horizontal lines in the displays. Results of the type shown in panel A indicate that the sequence is not methylated at the 5'CCGG site in each genomic location since the band is observed in equal intensity in both paired digests of every fraction. Results of the type shown in B in which the band is absent in each *HpaII* lane indicate concerted methylation at the site.

fragments of lower copy number. Preliminary estimates based on comparisons of the observed intensities of these bands with standards, suggests that the families forming bands in Figure 2 range in copy number from about 500 to 50,000 copies/haploid genome.

A second type of information derives from the use of *HpaII* and *MspI*. This allows one to inspect the repeated sequences which are resolved in the patterns for methylation at the 5'CCGG sequence. Since methylation at the internal cytosine in this site blocks cleavage by *HpaII* but not by *MspI*, the ratio of intensity of a band observed in the *HpaII* lane to that in the corresponding *MspI* lane provides a measure of the degree of this type of modification at this site in the class of repeated sequences forming a band. In the *Physarum* genome displayed in Figure 2 most families of repeated sequences were not detected in the *HpaII* digests and are therefore highly modified. A few examples of repeated sequences which are not heavily modified and are observed in the *HpaII* lanes are marked A in the figure. Examples of bands present only in the *HpaII* lane are marked B in the figure. Bands of

this type could be generated from repeated sequences in which all members of the repeat class present HpaII sites as 5'MCGG, where M denotes 5-methylcytosine.

One of the most important opportunities afforded by the use of the HpaII/MspI variant of the 2-D display technique is the opportunity to obtain information on what can be called concerted methylation of closely related or identical members of a class of repeated sequence. For example, in some instances a repeated sequence which resides at several different genomic locations may contain only a small number of cleavage sites for the enzymes used in the initial digestion of high molecular weight DNA (in this case BamHI). Cleavage at one or more sites within the repeat, and the sites in the DNA sequences which flank the repeat will produce a collection of fragments of various lengths all containing a segment of the repeated sequence bounded at one end by a segment of flanking DNA of various types and lengths (see Figure 3). During the electroelution process used to construct the two-dimensional displays, fragments with a repeated sequence at one end are fractionated by molecular length.²⁶ When the repeated DNA fragments contain a 5'CCGG which is concertedly modified, i. e. methylated at each genomic location in which it resides, then paired digestion of the DNA in each fraction with HpaII and MspI will produce identical fragments of the repeated sequence in every MspI lane of each pair. In terms of the geometry of the two-dimensional display, this will produce a horizontal line in the pattern with bands present in the MspI lane and absent from the HpaII lane of each paired digest (Figure 3B). A sequence which is maintained by cellular mechanisms in an unmodified state at many genomic locations can be termed concertedly unmodified. Sequences of this type will be cleaved by both HpaII and MspI, producing fragments of the repeat which are identical in length and autoradiographic intensity in both the HpaII and the MspI lane of each fraction. A horizontal line will again be produced in the two-dimensional display, but in this case both the HpaII and MspI lane of each fraction will contain bands produced by the repeat (see Figure 3A). The two-dimensional display of Physarum polycephalum DNA shown in Figure 2 illustrates these points. Repeated sequences which are concertedly modified are marked C in the figure. No concertedly unmodified sequences were observed in the Physarum pattern.

A comparison of the DNA from the clone DS-19 isolated before or after induction of globin with HMBA was performed using the two-dimensional display technique. No significant difference between the two patterns was observed, extending the results of the experiments shown in Figure 1. With the

resolution afforded by the two-dimensional display we were able to compare numerous repeated sequence classes for changes in modification. No changes were observed and we conclude that treatment with HMBA does not cause dramatic changes in the modification of non-satellite²⁷ repeated DNA in clone DS-19.

In contrast, the two-dimensional displays produced from normal mouse spleen DNA was quite different from those of the DS-19 DNA. A representative display of the DNA of mouse spleen is shown in Figure 4, and that of the HMBA treated DS-19 DNA is shown in Figure 5. The difference in overall level of modification first observed in the one-dimensional experiments shown in Figure 1 is extended to many homologous classes of repeated sequences with the two-dimensional displays shown in Figure 4 and 5. Four examples of repeated sequences occupying homologous positions in both two-dimensional displays are marked in the figure. A class of sequence marked A in both patterns is highly modified in spleen DNA, but nearly unmodified in the cell line. Two other examples (marked B) are seen weakly in the concertedly modified state in the spleen pattern but are prominent and concertedly unmodified in the DNA of the Friend cell line. A family of sequences which possess a low level of modification in both the spleen and the cell line is marked C in the figures.

It is also apparent from a comparison of the two figures, that there is a concentration of label in the low molecular weight region of the two-dimensional display from Friend cell DNA. We can not adequately explain this observation at this time, but we have observed that there is preferential labeling of a minor low molecular weight component present as only a small fraction of DNA from the cell line. This minor component is not observed in ethidium bromide stained gels of BamHI digests of DS-19 DNA unless large amounts of DNA are loaded (1.5 µg/lane). However, more than 50% of the label in autoradiographs of end-labeled digests is found in the low molecular weight region of such preparations.

DISCUSSION

We have studied methylation in the three types of DNA, that of Physarum polycephalum, that of a murine erythroleukemic cell line, and that of the tissues of normal mouse spleen from which this cell line was originally derived. Christman *et al.*²³ have reported that the total 5-methyl-cytosine content of DNA in the Friend cell line decreases by about 1.6% as a function of induction of differentiation. We, therefore, performed a series of experiments designed to detect changes in modification as a function of globin induction in clone DS-19 of the Friend cell line. HpaII and MspI cleavage

Figure 4. Two-Dimensional Display of the DNA of Balb/c Mouse Spleen DNA. Two-dimensional displays were prepared from high molecular weight mouse spleen DNA as described in the legend to Figure 1. Most repeated sequences displayed are highly modified at 5'CCGG. Repeated sequence classes marked A, B, and C occupy homologous positions in the two-dimensional displays of spleen DNA and Friend cell DNA. In spleen cell DNA repeated sequence classes marked A and B are heavily modified. The repeated sequence class marked C is weakly modified. Similar results were obtained with DBA/J2 DNA.

Figure 5. Two-Dimensional Display of the DNA of Friend Cells After Induction of Globin with Hexamethylene-Bis-Acetamide. Two-dimensional displays were prepared from high molecular weight Friend cell DNA as described in the legend to Figure 4, both before and after induction of globin with hexamethylene-bis-acetamide. No significant difference in the modification at 5'CCGG was observed when displays from induced and uninduced cells were compared. The pattern shown is markedly different from that of Balb/c spleen DNA shown in Figure 4. Most repeated sequence classes displayed in the pattern are not significantly modified at 5'CCGG. Repeated sequence classes marked A and B are not significantly modified in Friend cells, but are highly modified in spleen cells. The repeated sequence class marked C is weakly modified in each pattern.

frequencies did not change as a function of globin induction with HMBA, although more than 95% of the cells expressed globin after induction as shown by the benzidine staining method. We extended this comparison with the sequential digestion technique for the two-dimensional display of restriction fragments. With this method, we observed no significant changes as a function of globin induction in modification of those repeated sequences which were resolved. While the methods employed here are quite sensitive to changes at 5'CCGG, a change in total modification of only 1.6% might not have been detected.

These results were in marked contrast to the results obtained with these techniques when DNA of clone DS-19 was compared (either before or after induction with HMBA) with DNA from normal mouse spleen tissue. As seen in Table 1 the cleavage frequency for HpaII was about two-fold lower in normal spleen DNA than in DS-19 DNA, while the MspI cleavage frequency was about the same in both types of DNA. Although the specificities of HpaII and MspI are complex as pointed out by McClelland,²³ a difference in HpaII cleavage frequency indicates a difference in methylation at the internal cytosine in the 5'CCGG sequence. Thus clone DS-19 appears to possess only 50% of the methylation at this cytosine present in the spleen tissue from which it was derived. If the 5'CCGG tetramer is representative of total DNA modification in mouse, then we can conclude that total methylation at cytosine is 50% lower in DS-19 than it is in normal spleen.

Gruenbaum *et al.*³⁵ have recently reported that the methylase(s) of mouse ascites tumor preferentially modify cytosine in 5'CG sequences in purified ϕ X174 DNA. Thus, one explanation for the results we have obtained would be that the activity of one or more methylases of this type has been lost or modulated downward in clone DS-19. It should be pointed out that modulation of this type could be accomplished in many ways, some of which might involve chromatin proteins or structures which serve as cofactors for a methylase of the type studied by Gruenbaum *et al.*³⁵

Evidence from work with repeated sequences in eukaryotic DNA suggests that mechanisms exist by which many interspersed repeated sequences would be modified to the same extent at 5'CCGG independent of genomic location, a phenomenon which can be described as concerted modification. Evidence in support of this idea has been obtained by Whittaker *et al.*³⁶ for Physarum, Eden *et al.*³⁷ for chicken, and by Bird *et al.*³⁸ for sea urchin with the aid of Southern blotting. Smith *et al.*²⁷ have obtained similar results for mouse with two-dimensional displays. The results presented here with Physarum

simply confirm those of Whittaker *et al.*³⁶ and illustrate the utility of the two-dimensional technique. With this method interspersed repeated sequences which are concertedly modified, i. e. modified at each genomic location in which they reside, are displayed as horizontal lines present in MspI lanes but absent from HpaII lanes of the 2-D displays. The results with Physarum DNA show three good examples of this phenomenon, in that DNA bands present in horizontal lines in the displays are sharply confined to MspI lanes. This could be a result of the relatively small genome size of this organism, or it could be because the DNA of this organism comes from a single cell type.

When we compared the DNA of mouse spleen with that of the Friend cell line using this technique we were able to extend the results on the general difference in overall modification state at 5'CCGG, to sites which reside in specific classes of non-satellite repeated sequences. In at least two instances an interspersed repeated sequence class was shown to have lost modification in the cell line at many genomic locations.

As noted above, the results with Friend cell DNA compared to normal DBA/J2 mouse spleen DNA from which it was derived suggest that a modification system is active in normal spleen cells but less active in the Friend cell line. Given the natural history of the cell line, this apparent loss of function could be an effect of viral transformation. Alternatively, Friend erythroleukemia virus may selectively transform only a small population of cells in the spleen which maintain low levels of DNA modification before and after infection. It is also possible that the changes in modification we have observed are a property of clone DS-19 only, and are unrelated to viral transformation. Experiments of the type shown in Figure 1 with other clonal isolates could test the latter hypothesis. It appears that differences in DNA modification at 5'CCGG may not be a general property of mouse cancer cells, since Singer *et al.*³⁹ have shown that mouse teratocarcinoma DNA and normal tissue DNA are modified to about the same extent at this sequence. However, Cohen⁴⁰ has obtained evidence for decreased chromosomal DNA modification in mouse mammary tumors, Lypeyre and Becker⁴¹ have found that hypomethylation in rat hepatocellular carcinomas, and Kuhlman and Doerfler (see ref. 9) have also observed hypomethylation in adenovirus-12 induced hamster tumors. The results which we report here are consistent with the concept that hypomethylation is a necessary but not a sufficient condition for gene expression.⁴² Compounds like HMBA might then act to induce genes which have become potentiated for induction by the generally low level of methylation present in these cells.

Thus it would be of interest to test the hypomethylated cell types above for inducibility of specific genes with inducers like HMBA.

ACKNOWLEDGEMENTS:

We would like to thank Drs. Jim Hoch and Joel Gottesfeld for laboratory space, equipment, and many helpful suggestions, Drs. Harry Matthews and E. Morton Bradbury for the gift of *Physarum* cultures, and Karen Cooksey and Suzanne Winterstein for technical assistance. This work was supported by NIH fellowship 1F32 GM 07065-01 to S.S., and grant HL 21845 from the NIH to J.Y. This is publication number 2677 from the Department of Immunopathology.

†Present address: Penn Laboratories, Taipei, Taiwan

REFERENCES

1. Vanyushin, B.F., Tkacheva, S.D. and Belozersky, A.N. (1970). *Nature* **225**, 948-950.
2. Sinsheimer, R.L. (1955). *J. Biol. Chem.* **215**, 579-583.
3. Doskocil, J. and Strom, F. (1962). *Biochim. Biophys. Acta* **55**, 953-959.
4. Holliday, R. and Pugh, J.E. (1975). *Science* **187**, 226-232.
5. Riggs, A.D. (1975). *Cytogenet. Cell Genet.* **14**, 9-25.
6. Waalwijk, C. and Flavell, R.A. (1978). *Nucl. Acids Res.* **5**, 4631-4640.
7. Razin, A and Riggs, A.D. (1980). *Science* **210**, 604-610.
8. Wigler, M. (1981). *Cell* **24**, 285-286.
9. Doerfler, W. (1981). *J. Gen. Virol.* **57**, 1-20.
10. Friend, C., Priesler, H.D. and Scher, W. (1974). *Current Topics Devel. Biol.* **8**, 81-101.
11. Mantueil, S., Hamer, D.H. and Thomas, C.A., Jr. (1975). *Cell* **5**, 413-422.
12. Blin, N. and Stafford, D.W. (1976). *Nucl. Acids Res.* **3**, 2303-2308.
13. Ohta, Y., Tanaka, M., Terada, M., Miller, O.J., Bank, A., Marks, P.A. and Rifkind, R.A. (1976). *Proc. Natl. Acad. Sci., USA* **73**, 1232-1236.
14. Osborne, H.B., Bakke, A.C. and Yu, J. (1982). *Cancer Res.* **42**, 513-518.
15. Orkin, S.H., Haros, P. and Leder, P. (1975). *Proc. Natl. Acad. Sci., USA* **72**, 98-102.
16. Tsiftsoylou, A.S., Gusella, J.F., Volloch, V. and Housman, D.E. (1979). *Cancer Res.* **39**, 3849-3855.
17. Mohberg, J. and Rusch, H.P. (1969). *J. Bacteriol.* **97**, 1411-1418.
18. Hall, L., Turnock, G. and Cox, B.J. (1975). *Eur. J. Biochem.* **51**, 459-465.
19. Smith, S.S. and Thomas, C.A., Jr. (1981). *Gene* **13**, 395-408.
20. Botchan, M. et al. (1973). *Cold Spring Harb. Symp. Quant. Biol.* **38**, 383-395.
21. Hamer, D.H. and Thomas, C.A., Jr. (1975). *Chromosoma* **49**, 267-267.
22. Singer, J., Roberts-Ems, J. and Riggs, A.D. (1979). *Science* **203**, 1019-1021.
23. McClelland, M. (1981). *Nucl. Acids Res.* **9**, 5859-5865.
24. Potter, S.S. and Newbold, J.E. (1976). *Anal. Biochem.* **71**, 452-458.
25. Potter, S.S., Bott, K.F. and Newbold, J.E. (1977). *J. Bacteriol.* **129**, 492-500.
26. Potter, S.S. and Thomas, C.A., Jr. (1977). *Cold Spring Harbor Symp. Quant. Biol.* **42**, 1023-1031.

27. Smith, S.S., Reilly, J.G. and Thomas, C.A., Jr. (1981) in *Developmental Biology Using Purified Genes*. ICN-UCLA Symposia on Molecular Cloning and Cellular Biology, Brown, D. and Fox, C.F. Eds., Vol. 23, Academic Press, New York, pp. 635-646.
28. Evans, H.H. and Evans, T.E. (1970). *J. Biol. Chem.* 245, 6436-6441.
29. H., Evans, T.E. and Lethman, S. (1973). *J. Mol. Biol.* 74, 563-572.
30. Reilly, J.G., Braun, R. and Thomas, C.A., Jr. (1980). *FEBS Lett.* 116, 181-184.
31. Ehrlich, M. and Wang, R.Y.H. (1981). *Science* 212, 1350-1357.
32. Mohberg, J. and Rusch, H.P. (1971). *Exp. Cell. Res.* 66, 305-316.
33. Venderly, W.A. and Venderly, V.K. (1949). *Experientia* 5, 327-330.
34. Christman, J.K., Welch, N., Schoenbaum, B., Schneiderman, N. and Acs, G. (1980). *J. Cell Biol.* 86, 366-370.
35. Gruenbaum, Y., Cedar, H. and Razin, A. (1982). *Nature* 295, 620-622.
36. Whittacker, P.A. *et al.* (1981). *Nucl. Acids Res.* 9, 801-814.
37. Eden, F.C., Musti, A.M. and Solieski, D.A. (1981). *J. Mol. Biol.* 148, 129-151.
38. Bird, A.P., Taggart, M.H. and Smith, B.A. (1979). *Cell* 17, 889-901.
39. Singer, J., Singer, J., Roberts-Ems, J., Luthardt, F.W. and Riggs, A.D. (1979). *Nucl. Acids Res.* 7, 2369-2385.
40. Cohen, J.C. (1980). *Cell* 19, 653-662.
41. Lapeyre, J.N. and Becker, F.F. (1979). *Biochem. Biophys. Res. Commun.* 87, 698-705.
42. Van der Ploog, L.T.H. and Flavell, R.A. (1980). *Cell* 19, 947-958.