Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6055–6059, September 1985
Biochemistry

Perturbation of maintenance and *de novo* DNA methylation *in vitro* by UVB (280–340 nm)-induced pyrimidine photodimers

(UV carcinogenesis/DNA methyltransferase/cyclobutane pyrimidine dimers)

FREDERICK F. BECKER, PAULA HOLTON, MATHUROS RUCHIRAWAT, AND JEAN-NUMA LAPEYRE

Section of Experimental Pathology, Department of Pathology, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston, TX 77030

Communicated by Sidney Weinhouse, May 17, 1985

ABSTRACT **The effect of pyrimidine photodimers on transmethylation reactions catalyzed by a highly purified rat liver DNA (cytosine-5-)-methyltransferase (EC 2.1.1.37) that exhibits maintenance and *de novo* methylation activities was studied *in vitro*, using the viral substrates M13 mp9 replicative form (RF) DNA and the hemimethylated analog formed from primed synthesis of phage DNA in the presence of 2′-deoxy-5-methylcytidine 5′-triphosphate. These DNAs were irradiated with UVB (280–340 nm) at 900–3600 J/m^2 in the presence of the triplet-state sensitizers acetone or 3-dimethylaminopropiophenone. Under these conditions of irradiation, which approximate solar UV, pyrimidine cyclobutane photodimers were introduced without producing any evidence of single-strand breaks or alkali-sensitive sites [i.e., no (6-4)pyrimidine-pyrimidone photoproducts]. This was confirmed by gel analysis, a T4 UV endonuclease nicking assay specific for cyclobutane-type dimers, and HPLC analysis of the photoproducts. The methylation of irradiated templates by DNA methyltransferase was inhibited in an approximately linear fashion as a function of increasing UVB dose. This inhibition was correlated with the number of lethal photoproducts detected by the simultaneous measurement of the surviving fraction of infectious phage DNA. For approximately the same number of pyrimidine cyclobutane photoproducts introduced, *de novo* methylation activity was ≈2-fold more sensitive than the maintenance mode of methylation. The ability of these putatively carcinogenic, pyrimidine photoproducts to inhibit DNA methylation suggests a common mechanism of action with several chemical carcinogens that are known to modify bases.**

The perpetuation of a specific pattern of genomic methylation may be necessary for maintenance of the phenotype of differentiated cells. Evidence for this comes from a number of studies that indicate an inverse correlation between methylation of eukaryotic and viral genes and their expression (summarized and reviewed in refs. 1–3) and more recent microinjection and transfection experiments (4–7). Although certain genes may not be methylation-sensitive (8), a large number of RNA polymerase II-transcribed genes appear to be, as indicated by studies using 5-azacytidine or its deoxy analog. These drugs cause suicide inhibition of DNA methyltransferases (9, 10) and exert profound effects on differentiation and gene expression in cells (reviewed in ref. 11).

DNA (cytosine-5-)-methyltransferase (EC 2.1.1.37) may have a dual catalytic role. It is required for methylation of hemimethylated sites shortly after DNA replication to perpetuate a specific genomic DNA methylation pattern (12, 13), but it is also likely to operate in a *de novo* initiating mode during development (14) and to be responsible for delayed methylation (15), parental-strand methylation (16), and possibly X chromosome inactivation (17). In addition, this initiating activity may provide a mechanism for the cell to preserve its pattern of methylation, after partial loss of methylated cytosines through damage to both strands of DNA, and to operate in the event that a new site is to be methylated.

Holliday (18) originally described multiple modes by which altered methylation of DNA might play a role in carcinogenesis; much support for this hypothetical role has appeared recently (reviewed in ref. 19). For example, Lapeyre and Becker (20) have found a striking undermethylation of nuclear DNA from putative, premalignant liver lesions induced by chemical carcinogens and in the cancers that result. The effect of interaction between chemical carcinogens and DNA on subsequent methylation has been examined frequently. Several problems exist in the interpretation of the results, among which are the complexity of the altered bases thus produced and the lack of strong evidence for a relationship between the chemical adducts and the carcinogenic process.

Ultraviolet radiation is known to have lethal, mutagenic, and tumorigenic effects on cells (21). The precise molecular lesions and repair processes involved in photocarcinogenesis are not known. However, one class of photoproduct, the cyclobutane pyrimidine dimers, is known to exert all the above effects (22). Hart *et al.* (23) have provided evidence that cyclobutane photodimers produced by 254 nm UV radiation induce tumors in irradiated Amazon mollies, since photoreactivation with >360 nm light, which specifically converted such photoproducts to monomers, decreased the yield of tumors by >90%. Further, it is probable that the UVB range (280–340 nm), which encompasses the major UV component of sunlight (295–315 nm), is the major contributor to the induction of skin cancers in man (24). Most of the shorter-wavelength UV (<280 nm) is filtered by the ozone layer, whereas longwave UV (>360 nm) exerts weak biological effects because of its lower quantum energy and its ability to photoreactivate cyclobutyl-type dimers (25).

In mice, UVB, with or without topical application of photosensitizers, is known to induce skin cancers (26, 27). Furthermore, UV irradiation of cells can lead to removal of specific methylated bases during subsequent rounds of DNA replication, perhaps as a consequence of aberrant repair (28). UV exposure has thus been used recently to activate the transcription of the erstwhile quiescent metallothionine gene, which, based on 5-azacytidine-reactivation experiments, has been presumed to be under methylation control (29). These considerations led us to test whether UVB-induced photodimers are capable of perturbing enzymatic DNA methylation *in vitro* under specific conditions of UVB irradiation in which no DNA single-strand breaks, or alkali-

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.

Abbreviations: DMAP, 3-dimethylaminopropiophenone hydrochloride; UVB, radiation of wavelength 280–340 nm; RF, replicative forms.

sensitive lesions, such as (6-4)pyrimidine-pyrimidone [(6-4)Pyr-Pyo] photoproducts, are detected (30).

MATERIALS AND METHODS

DNA Methyltransferase Preparation and Assay. Rat liver DNA cytosine methyltransferase was purified as described (31) to fraction IV. The methyltransferase isolated by this procedure is ≈1500-fold purified, contains both initiating and maintenance methylation functions, has a molecular weight of 280,000 by gel filtration, and preferentially methylates double- over single-stranded DNA and hemimethylated over unmethylated double-stranded DNA (32). The standard assay in 200 μl contained 5 μM *S*-adenosyl-L-[*methyl*-^{3}H]methionine (ICN, 9 Ci/mmol; 2 μCi per assay; 1 Ci = 37 GBq), various amounts of fraction IV enzyme, and DNA in 20 mM Tris Cl, pH 7.4/25 mM NaCl/0.5 mM dithiothreitol. Maintenance-type methylation activity was measured with 0.5 μg of hemimethylated mp9 double-stranded phage DNA circles (33). Initiation-type methylation activity was measured with M13 mp9 replicative form DNA. The efficiency of irradiated templates in supporting methylation was defined as the capacity of a constant amount of methyltransferase to methylate these various templates over the initial 30-min linear phase of the reaction at 37°C. The reactions were stopped and processed according to Chan *et al.* (34).

DNA Templates. Single-stranded phage DNA and replicative forms (RFs) of the M13 vector mp9 were isolated as described (35). Primer-extended, hemimethylated circles were synthesized with DNA polymerase I Klenow fragment (New England Biolabs) and 2′-deoxy-5-methylcytidine 5′-triphosphate (dm^{5}CTP; P-L Biochemicals) in place of dCTP, using the 17mer M13 universal primer analogously to the procedure described by Grunenbaum *et al.* (13) for ϕX174 hemimethylated DNA. After annealing of the primer, the reaction was started at 14°C for 10 min and then allowed to proceed at 29°C to give a full-length, complementary, hemimethylated strand. The hemimethylated phage circles were deproteinized by two phenol/isoamyl alcohol/chloroform (1:0.98:0.02, vol/vol) extractions and then were ethanol-precipitated. RF were isolated from *Escherichia coli* JM101 cells 45 min after infection by the procedure of Messing *et al.* (36). Phage DNA and RF infectivity was determined from the titer of blue plaques obtained by $CaCl_2$-mediated transformation of competent JM101 cells. Except for hemimethylated templates, for which the infectivity is ≈2% that for unmethylated RF DNA (37), duplicate aliquots (1–2 ng) which yielded ≈2000 plaques per dish were taken from the samples immediately after irradiation. The samples were subsequently analyzed for the ability to serve as efficient templates for methylation *in vitro*. For determination of the surviving infectivity of the hemimethylated DNAs, 100 ng was removed in order to get a sufficient number of plaques (600–1000).

DNA Irradiation and Sensitizers. DNA was irradiated at room temperature with a 300- to 320-nm-output lamp (Photodyne, catalog no. 3-440A) at a distance of 50 cm in the presence of oxygen. The samples in quartz cuvettes were shielded from <300 nm light with a 1 cm thick layer of 0.02% $CoCl_2$ in dimethylformamide. The fluence of UVB was determined with a calibrated longwave UV meter (J-221, Ultraviolet Products, San Gabriel, CA), and the specific output was 0.5 W/m^2 with the 300-nm-cutoff filter in place. The triplet-state photosensitizer 3-dimethylaminopropiophenone hydrochloride (DMAP) (Aldrich) was used at a final concentration of 12 mM (A_{313} = 0.84) (38), and HPLC-grade acetone was used at 10% (vol/vol; A_{313} = 0.22) (39). These compounds have triplet-state excitation energies (E_t) of 26,500 cm^{-1} (38) and 28,200 cm^{-1} (39), respectively. To remove the cationic monoamine sensitizer DMAP, the DNA samples were passed over a 1-cm bed of Dowex AG 50W-X8 Na$^+$ form (Bio-Rad) and then recovered by ethanol-precipitation.

Photoproduct Determinations. The relative number of UVB-induced photoproducts was quantitated from the fraction of surviving infectious, double-stranded, hemimethylated phage DNA circles or unmethylated RFs as described under DNA templates. Specific formation of pyrimidine cyclobutane dimers was assessed using a DNA nicking assay with purified T4 UV endonuclease (40). The assays with the purified enzyme were performed by incubating irradiated mp9 RF DNA (0.5 μg) for 15 min at 37°C in 10 mM Tris Cl, pH 7.4/0.1 M NaCl/10 mM EDTA with nuclease-free bovine serum albumin (100 μg/ml) and 2 μg of T4 UV endonuclease (generously provided by N. Duker) (41). The conversion of form I superhelical molecules to relaxed circles and linear forms was assessed by electrophoresis in 1% agarose gels. Cyclobutane thymine-thymine and thymine-cytosine dimers and the corresponding (6-4)Pyr-Pyo photoproducts were generated by irradiation of a solution of dTpdT and dTpdC dinucleotides (P-L Biochemicals), respectively, at a dose of 10^5 J/m^2 with a germicidal UV lamp (Sylvania G30T8) with peak output at 254 nm, according to Franklin *et al.* (30). These samples were hydrolyzed in concentrated formic acid and then used as standards during HPLC analysis of UVB-treated samples. The resultant bases and photoproducts were separated by reversed-phase HPLC on an Alltech (Deerfield, IL) 10-μm C_{18} reversed-phase column (0.625 cm × 25 cm); elution was with buffer A (75 mM potassium phosphate, pH 4.5) for 5 min and then with a linear gradient from 100% buffer A to 40% buffer A/60% buffer B (75 mM potassium phosphate, pH 4.5/40% methanol). Spectroscopic measurements at various wavelengths were made with a Kratos M773 variable wavelength detector in order to identify cyclobutyl photodimers (at 230 nm) and (6-4)Pyr-Pyo products (at 300–310 nm) and their relative elution positions (30).

To determine the distribution of pyrimidine photoproducts under conditions of the methylation assay, primer-extended, second-strand synthesis was carried out with DNA polymerase Klenow fragment and [*methyl*-^{3}H]dTTP (ICN, 50 Ci/mmol) as described above for the formation of hemimethylated templates. After irradiation and removal of the bound DMAP, the DNA samples were hydrolyzed for 30 min in concentrated formic acid at 175°C (42). Authentic standards of thymine-thymine and thymine-cytosine photodimers and (6-4)Pyr-Pyo product were coinjected in each HPLC run to mark the position of the eluting products, which were monitored at 230 nm and 310 nm. The tritium dpm distribution was normalized for total dpm recovered by subtraction of a control distribution, for which an equal amount of labeled but unirradiated mp9 DNA was processed in the same fashion. The resultant data were expressed as the parts per million (ppm) of [^{3}H]methyl-labeled moieties in each fraction.

RESULTS

Characterization of Irradiated DNA Templates. The integrity of DNA templates after UV irradiation in the UVB range (280–340 nm) was examined as a function of UV fluence and the type of triplet-state photodimer sensitizer that was used. The mp9 RF DNA was UVB-irradiated (0–3600 J/m^2) in the presence of either 10% acetone or 12 mM DMAP and analyzed by electrophoresis for UV-induced single-strand breaks (Fig. 1*A*). No change in the proportion of form I superhelical molecules was detected, demonstrating that photonicking by this UV energy in the presence of the triplet-state sensitizers DMAP or acetone could not be detected and cannot account for any effects on *in vitro* DNA methylation. Fig. 1*B* shows the results obtained when the same experiment and conditions described for Fig. 1*A* were

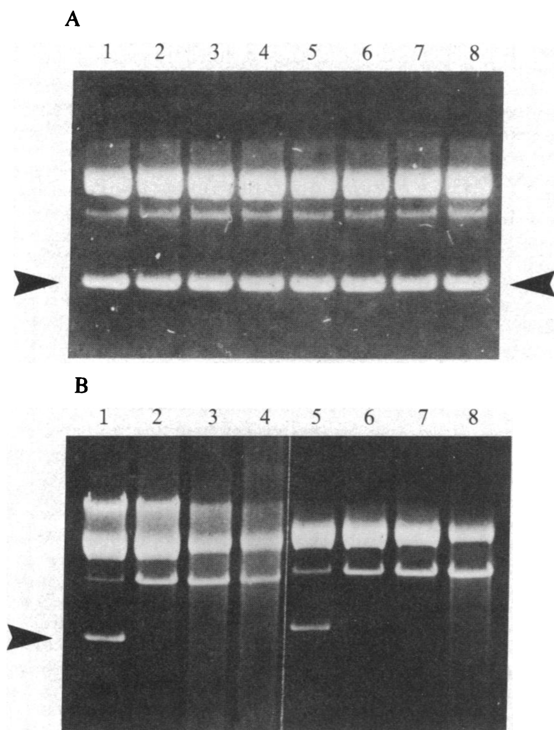

FIG. 1. (A) Effect of dose of UVB irradiation on mp9 RF DNA integrity after photosensitization with 10% acetone (lanes 1–4) or with 12 mM DMAP (lanes 5–8). Lanes: 1 and 5, unirradiated control; 2 and 6, 900 J/m^2; 3 and 7, 1800 J/m^2; 4 and 8, 3600 J/m^2. Arrowheads denote form I superhelical molecules. (B) T4 UV endonuclease assay for pyrimidine cyclobutane dimers as a function of UVB fluence. Lanes contained samples as in A except that the samples were incubated with 2 μg of T4 UV endonuclease for 15 min at 37°C before electrophoresis.

followed except that the mp9 RF templates were then incubated with purified T4 UV endonuclease and the proportion of superhelical form I molecules converted to relaxed circles and linear molecules was determined. T4 UV endonuclease recognizes pyrimidine cyclobutane dimers and catalyzes cleavage of the phosphodiester bond at the 5′ adjacent nucleotide by breaking the N-glycosydic bond and incising the resultant apyrimidinic site (43). The complete disappearance of form I molecules at the lowest dose used (900 J/m^2, lanes 2 and 6; compare to unirradiated molecules in lanes 1 and 5) indicates that all molecules have been cleaved at least once and, therefore, at least one cyclobutane pyrimidine dimer per molecule was induced at the lowest fluence used.

Quantitation of the number of UV-induced lethal photoproducts introduced by various photosensitizers as a function of UVB fluence is shown in Fig. 2. The fraction of surviving infectivity of unmethylated RF molecules and their hemimethylated, double-stranded analogs was measured in *E. coli* JM101. This host is not defective for cyclobutyl dimer repair catalyzed by the *uvrABC* genes and, therefore, the plaque titer underestimates the number of lethal UV-induced lesions. The plaque titer gives an estimate of the relative number of photoproducts present in the substrate DNAs. The yield of irradiated mp9 RF molecules was reduced 70% by UVB-irradiation at 3600 J/m^2, whereas in the presence of the triplet-state photosensitizers acetone or DMAP, this titer was further reduced to 5% and 1% of the original total, respectively. Similarly, mp9 phage molecules whose second strand was synthesized by primer-extension using dm^{5}CTP in place of CTP showed approximately the same percentage of surviving molecules at the highest dose used (i.e., 1–3% of the

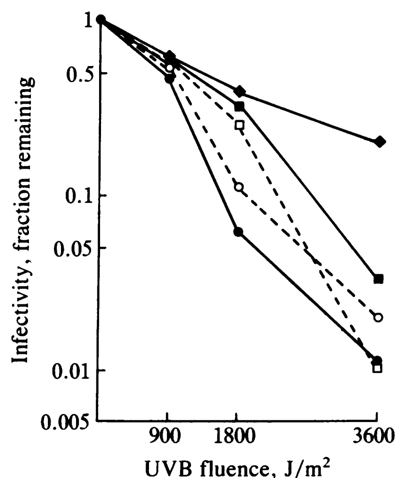

FIG. 2. Surviving infectivity of mp9 RF and hemimethylated mp9 templates as a function of UVB dose. Results are shown for mp9 RF not photosensitized (◆); mp9 RF, 12 mM DMAP (■); mp9 RF, 10% acetone (□); mp9 hemimethylated DNA, 12 mM DMAP (●); and mp9 hemimethylated DNA, 10% acetone (○).

total) and a dose-dependent decrease in plaque titer with increasing UVB fluence, even though 50-fold higher input of DNA had to be used to perform this assay (37).

Characterization of UVB-Induced Photoproducts. Double-stranded mp9 molecules were synthesized in the presence of [*methyl*-^{3}H]dTTP and purified prior to irradiation. After 0, 30, and 120 min of UVB irradiation at 0.5 W/m^2 and repurification to remove the sensitizers, equal amounts of labeled molecules were hydrolyzed to bases with concentrated formic acid and spiked with thymine-thymine and thymine-cytosine photodimer standards. At the highest fluence (3600 J/m^2), thymine-cytosine and thymine-thymine cyclobutane dimers were found at 2200 and 8200 ppm, respectively, for acetone sensitization, and 4200 and 5600 ppm, respectively, for DMAP sensitization. There was <100 ppm of detectable (6-4)Pyr-Pyo product at highest fluence of UVB. These results are in accord with previous findings on (6-4)Pyr-Pyo photoproducts formation after UVB irradiation (44). However, under these conditions a significant proportion of thymine-cytosine dimers were formed (20% for acetone and nearly 40% for DMAP), in contrast to irradiation at 254 nm, for which the production of thymine-thymine dimers greatly exceeds that of thymine-cytosine dimers (45). There was no significant difference in distribution and type of photoproducts formed at 3600 J/m^2 from those formed at 900 J/m^2 (data not shown).

Action of Rat Liver DNA Cytosine Methyltransferase on Irradiated DNAs. Primer-extended M13 phage DNA synthesized in the presence of dm^{5}CTP was used to measure maintenance methyltransferase activity, while the same template primer extended with dCTP served as a substrate to measure *de novo* activity. These substrates distinguish *de novo* from maintenance methylation activity and permit the use of the T4 UV endonuclease assay for pyrimidine photodimer formation and their quantitation from infectivity measurements. When the methyltransferase functions in a maintenance mode using hemimethylated DNA rather than (double-stranded) unmethylated DNA as substrate, a much higher rate of methyl-group transfer is effected even though the concentration of potential sites for methylation is halved (Fig. 3). This type of catalytic behavior has been observed for several other types of preparations of this methyltransferase (reviewed in ref. 32). During the first 30 min of the reaction, where the rate of transfer is essentially linear, this preparation has about a 10-fold selectivity for methylating sites in

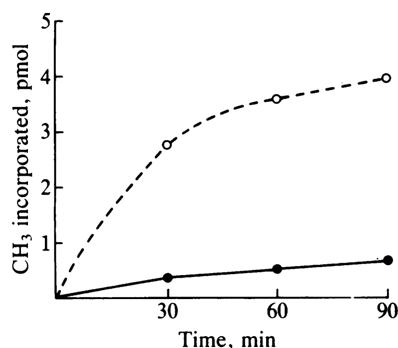

FIG. 3. Kinetics of maintenance and initiation-type methylation catalyzed by rat liver DNA cytosine methyltransferase. ○, Hemimethylated mp9 (primer-extended second strand containing 5-methylcytosine in place of cytosine); ●, unmethylated mp9 RF.

hemimethylated M13 DNA over sites in unmethylated double-stranded M13 DNA. The efficiency of rat liver methyltransferase-catalyzed methylation of unphotosensitized mp9 RF and of acetone or DMAP-sensitized molecules and their hemimethylated analogs was determined as a function of UVB fluence (Fig. 4). The methylation efficiency for unphotosensitized, irradiated mp9 RF DNA in the *de novo* mode was higher than for the same DNAs photosensitized with either acetone or DMAP, as expected from the level of lethal photoproducts introduced (Fig. 2). When the enzyme functions in the maintenance mode, its sensitivity to inhibition by UVB irradiation is less than that for *de novo* methylation of unmethylated DNA, for approximately the same amount of photoproducts in the respective templates [HPLC analysis of irradiated samples (not shown); Fig. 2.].

The observed inhibition of methylation in the initial phase of the reaction should parallel a decrease in V_{max} of the methyltransferase on these various templates. This was verified by kinetic analysis of the enzyme's action on DMAP-photosensitized mp9 RF irradiated at 3600 J/m^2 compared to its unirradiated control. UVB irradiation at 3600 J/m^2 results in a 41% reduction in V_{max}, which is in good agreement with the calculated inhibition from initial-velocity template efficiency measurements (Fig. 4). Furthermore, a significant decrease in the affinity of the enzyme for the irradiated templates occurred, as the K_m decreased from $(4.0 \pm 0.09) \times 10^{-10}$ M to $(3.67 \pm 0.14) \times 10^{-10}$ M (DNA nucleotide units)

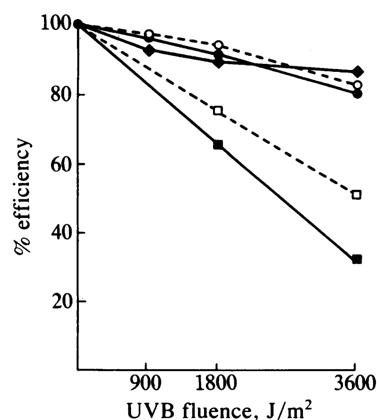

FIG. 4. Efficiency of methylation of hemimethylated and unmethylated substrates as a function of UVB dose. Relative methylation by rat liver DNA methyltransferase is shown for mp9 RF not photosensitized (◆); mp9 RF, 12 mM DMAP (■); mp9 RF 10% acetone (□); hemimethylated mp9, 12 mM DMAP (●); and hemimethylated mp9, 10% acetone (○).

for the photosensitized, irradiated sample. Although similar kinetic determinations on the hemimethylated templates would be of interest, these templates do not obey Michaelis–Menten kinetics. Nonetheless, the data in Fig. 4 suggest that an impairment of methyltransferase activity would be reflected by a substantial decrease in V_{max}, to about 20% of that for the enzyme operating on irradiated DNA with hemimethylated sites.

DISCUSSION

A number of lines of investigation support the hypothesis that UV-induced photoproducts, and in particular the cyclobutane pyrimidine dimers, have a direct role in carcinogenesis. These include the proclivity of cells defective in the repair of these DNA alterations to malignant transformation (46), the reversal of tumorigenicity of UV-irradiated cells by photoreactivation (23), and the strong correlation between the development of cancer in skin with prior chronic UVB exposure (47). Smith and Paterson have provided evidence that UVB- and far-UV-induced pyrimidine dimers are the lesions responsible for lethality to normal cells and cells from patients with Bloom syndrome or xeroderma pigmentosum, whereas the role of DNA single-strand breaks appears to be minor (48). A link between UV irradiation, defective postreplication repair, and altered methylation has been reported: UV irradiation was shown to lead to specific demethylation events during subsequent rounds of replication (28). As a consequence of aberrant repair or repair methylation, UV irradiation has also been used to activate the transcription of a quiescent metallothionine gene, which, based on 5-azacytidine-reactivation experiments, is thought to be under methylation control (29).

The experiments reported herein using a highly purified DNA cytosine methyltransferase were instigated to determine whether, under conditions of UVB irradiation that closely approximate solar UV exposure, the resultant DNA photoproducts could inhibit either maintenance or *de novo* methylase functions *in vitro*. Conditions of UVB irradiation were selected that eliminated the potential effects of altered DNA integrity resulting from nicking and alkali-sensitive lesions attributed to (6-4)Pyr-Pyo photoproducts (30). Under these conditions, UVB irradiation in the 900–3600 J/m^2 dose range produced principally thymine-thymine and thymine-cytosine cyclobutyl photodimers (in the 2000–8000 ppm range); <100 ppm (6-4)Pyr-Pyo photoproducts were elicited at the highest dose tested. A decrease in infectivity and corresponding inhibition of methylation correlated with an increase in the concentration of thymine-thymine and thymine-cytosine photodimers (cytosine-cytosine dimers were not measured). For M13 substrates *in vitro*, initiation-type methylation was more sensitive to inhibition by UVB-induced pyrimidine photoproducts than maintenance-mode methylation. This difference may be significant because this form of methyltransferase activity would be involved in the restoration of the methylation pattern after DNA repair, which would be especially important for restoration of a specific pattern of methylation after carcinogen exposure. Although the M13 substrates are not identical to eukaryotic DNA, these results are interpreted to suggest that UV photoproducts could alter methylation patterns *in vivo*.

The mechanism by which the inhibition of enzymatic methylation occurred has not been identified. These experiments show that it may be related to the participation of a cytosine in a CpG methylation site with a 5′-adjacent pyrimidine in a photodimerization event. The possibility of a 5-methylcytosine forming a pyrimidine cyclobutyl dimer with an adjacent pyrimidine exists but has not been detected to date; such dimers could affect maintenance methylation. Excimer production and photodimerization of 5-methylcyto-

sine are highly probable events, since it is the only base normally fluorescent under physiological conditions (49). *In vivo* inhibition may also occur when the daughter-strand cytosine in a hemimethylated site is photodimerized during DNA replication. This possibility is likely because UVB irradiation greatly increased the yield of thymine-cytosine relative to thymine-thymine dimers, as reported here and by Ellison and Childs (45). It is also possible that pyrimidine dimers in the vicinity of a CpG methylation site upset the maintenance or *de novo* activities or both.

Although evidence for an obligate role of a DNA methylation alteration in carcinogenesis remains circumstantial, our findings strengthen the possible relationship, since many chemical carcinogens have been determined to alter DNA methylation *in vitro* (31, 32, 50) and specific sites of genomic methylation in cells (51, 52). In numerous cases, this effect is manifested in alterations of genomic 5-methylcytosine levels, indicating a severe disturbance by chemical carcinogens of the regulation of genomic methylation (19, 20, 50–53). Whether such inhibition or perturbation of enzymatic methylation would result in a cascade of genetic aberrations and influence the regulation of genes controlling programs of differentiation or, more specifically, allow activation of oncogenes is hypothetical.

This investigation was supported by Grants CA31487 and CA20657 awarded by the National Cancer Institute. Support was also received from Conoco, Inc., which provided M.R. with a Fellowship in Toxicology.

1. Doerfler, W. (1983) *Annu. Rev. Biochem.* **52**, 92–124.
2. Felsenfeld, G. & McGhee, J. (1982) *Nature (London)* **296**, 602–603.
3. Razin, A. & Friedman, S. (1981) *Prog. Nucleic Acids Res. Mol. Biol.* **25**, 33–52.
4. Vardimon, L., Kressmann, A., Cedar, H., Maechler, M. & Doerfler, W. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1073–1077.
5. Kruczek, I. & Doerfler, W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7586–7590.
6. Simon, D., Stuhlmann, H., Jahner, D., Wagner, H., Werner, E. & Jaenisch, R. (1983) *Nature (London)* **304**, 275–277.
7. Busslinger, M., Hurst, J. & Flavell, R. A. (1983) *Cell* **34**, 197–206.
8. McKeon, C., Ohkubo, H., Pastan, I. & de Crombrugghe, B. (1982) *Cell* **29**, 203–210.
9. Creusot, F., Acs, G. & Christman, J. K. (1982) *J. Biol. Chem.* **257**, 2041–2048.
10. Santi, D. V., Garrett, C. E. & Barr, P. J. (1983) *Cell* **33**, 9–10.
11. Riggs, A. D. & Jones, P. A. (1983) *Adv. Cancer Res.* **40**, 1–30.
12. Bird, A. P. (1978) *J. Mol. Biol.* **118**, 49–60.
13. Grunenbaum, Y., Cedar, H. & Razin, A. (1982) *Nature (London)* **295**, 620–622.
14. Razin, A., Webb, C., Szyf, M., Yisraeli, J., Rosenthal, A., Naveh-Many, T., Sciaky-Gallili, N. & Cedar, H. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2275–2279.
15. Woodcock, D. M., Adams, J. K., Allan, R. G. & Cooper, I. A. (1983) *Nucleic Acids Res.* **11**, 489–499.
16. Hilliard, J. K. & Sneider, T. W. (1975) *Nucleic Acids Res.* **2**, 809–819.
17. Mohandas, T., Sparkes, R. S. & Shapiro, L. J. (1981) *Science* **211**, 393–396.
18. Holliday, R. (1979) *Br. J. Cancer* **40**, 513–521.
19. Nyce, J., Weinhouse, S. & Magee, P. N. (1983) *Br. J. Cancer* **48**, 463–475.
20. Lapeyre, J. N. & Becker, F. F. (1979) *Biochem. Biophys. Res. Commun.* **87**, 698–705.
21. Setlow, R. B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3363–3366.
22. Webb, R. B. (1978) in *Photochemical and Photobiological Reviews*, ed. Smith, K. C. (Plenum, New York), Vol. 2, pp. 169–261.
23. Hart, R. W., Setlow, R. B. & Woodhead, A. D. (1977) *Proc. Natl. Acad. Sci. USA* **12**, 5574–5578.
24. Parrish, J. A., Anderson, R. R., Urbach, F. & Pitts, D. (1978) *UVA* (Plenum, New York).
25. Setlow, R. B. & Carrier, W. L. (1966) *J. Mol. Biol.* **17**, 237–254.
26. Ley, D. D., Sedita, B. A., Grube, D. D. & Fry, R. J. (1977) *Cancer Res.* **37**, 3243–3248.
27. Epstein, J. H. (1984) *J. Natl. Cancer Inst.* **72**, 383–385.
28. Kastan, M. B., Gowans, B. J. & Lieberman, M. W. (1982) *Cell* **30**, 509–516.
29. Lieberman, M. W., Beach, L. R. & Palmiter, R. D. (1983) *Cell* **35**, 207–214.
30. Franklin, W. A., Lo, K. M. & Haseltine, W. A. (1982) *J. Biol. Chem.* **257**, 13535–13543.
31. Ruchirawat, M., Becker, F. F. & Lapeyre, J. N. (1984) *Nucleic Acids Res.* **12**, 2257–3371.
32. Lapeyre, J.-N., Ruchirawat, M. & Becker, F. F. (1985) in *Progress in Non-Histone Protein Research*, ed. Bekhor, I. (CRC, West Palm Beach, FL) Vol. 1, pp. 167–190.
33. Wang, R. Y.-H., Huang, L.-H. & Ehrlich, M. (1984) *Nucleic Acids Res.* **12**, 3473–3490.
34. Chan, J. Y. H., Ruchirawat, M., Lapeyre, J.-N. & Becker, F. F. (1983) *Carcinogenesis* **4**, 1097–1105.
35. Messing, J. & Vieira, J. (1982) *Gene* **19**, 269–276.
36. Messing, J., Gronenborn, B., Mullerhill, B. & Hofschneider, P. N. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3642–3646.
37. Wang, R. Y.-H., Shenoy, S. & Ehrlich, M. (1984) *J. Virol.* **49**, 674–679.
38. Meistrich, M. L., Lamola, A. A. & Gabbay, E. (1970) *Photochem. Photobiol.* **11**, 169–178.
39. Fisher, G. J. & Johns, H. E. (1976) in *Photochemistry and Photobiology of Nucleic Acids*, ed. Wang, S.-Y. (Academic, New York), Vol. 1, pp. 225–294.
40. Friedberg, E. C., Ganesan, A. K. & Seawell, P. C. (1980) *Methods Enzymol.* **65**, 193–201.
41. Duker, N. J., Davies, W. A. & Hart, D. M. (1981) *Photochem. Photobiol.* **34**, 191–195.
42. Carrier, W. L. & Setlow, R. B. (1970) *Methods Enzymol.* **13**, 230–237.
43. Radany, E. H. & Friedberg, E. C. (1980) *Nature (London)* **286**, 182–185.
44. Rahn, R. O. & Patrick, M. H. (1976) in *Photochemistry and Photobiology of Nucleic Acids*, ed. Wang, S.-Y. (Academic, New York), Vol. 2, 97–145.
45. Ellison, M. J. & Childs, J. D. (1981) *Photochem. Photobiol.* **34**, 465–469.
46. Cleaver, J. E. & Bootsma, D. (1975) *Annu. Rev. Genet.* **9**, 19–38.
47. Blum, H. F. (1959) *Carcinogenesis by Ultraviolet Light* (Princeton University Press, Princeton, NJ).
48. Smith, P. J. & Paterson, M. C. (1982) *Photochem. Photobiol.* **36**, 333–343.
49. Gill, J. E. (1970) *Photochem. Photobiol.* **11**, 259–269.
50. Boehm, T. L. S. & Drahovsky, D. (1983) *Recent Results Cancer Res.* **84**, 212–225.
51. Boehm, T. L. J., Grunberger, D. & Drahovsky, D. (1983) *Cancer Res.* **43**, 6066–6071.
52. Kuo, M. T., Iyer, B., Wu, S. R., Lapeyre, J.-N. & Becker, F. F. (1984) *Cancer Res.* **44**, 1642–1647.
53. Lapeyre, J.-N., Walker, M. J. & Becker, F. F. (1981) *Carcinogenesis* **2**, 873–876.