UV-induced photoproducts of 5-methylcytosine in a DNA sequence context

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Received December 10, 1987; Revised and Accepted March 16, 1988

ABSTRACT

In order to detect possible m^5C photoproducts, highly purified rat liver DNA-cytosine methyltransferase was used to specifically generate m^5C with a radioactive methyl group. When these DNAs were subjected to a large dose (10 kJ/m^2) of 254 nm or 302 nm ultraviolet light (UVB) to enhance the yield, two labeled photoproducts were detected and isolated by reverse phase HPLC after formic acid hydrolysis. Further studies using acetone as a triplet state sensitizer and UVB irradiation suggested that photoproduct II was activated via a triplet state while the more polar photoproduct I was not. Photoreversion of the purified photoproducts with 10 kJ/m^2 254 nm light demonstrated the following reactions: Photoproduct I regenerated m^5C , while photoproduct II is split and regenerated m^5C and photoproduct I. These results suggest that photoproduct I is monomeric while photoproduct II dimeric, and from the latter's elution position possibly a cyclobutyl type dimer arising from a reaction with an adjacent cytosine. Using $d[TTG]$ and $d[Cm^5CG]$ as models of typical sequences, irradiation with 10 kJ/m^2 254 nm or 302 nm, respectively, gave rise to a small component having altered mobility in sequencing gels. The altered mobility trinucleotides were resistant to degradation by PI and micrococcal nucleases as expected from photodimerization of the pyrimidine bases. Furthermore, oligonucleotide substrates containing m^5C were synthesized and shown to be susceptible to T4 endonuclease V action at locations consistent with $d[Cm^5C]$ photodimer formation when irradiated in the UVB range.

INTRODUCTION

Ultraviolet (UV) light is known to produce several classes of pyrimidine photoproducts in DNA, namely dimeric cyclobutyl and 6-4'-pyrimidin-2'-one-pyrimidine photoproducts in addition to monomeric photohydrates and peroxides (1,2). Although experiments based on excited states in the transition of 5-methylcytosine (m^5C) should not preclude the production of known types of pyrimidine photoproducts (3); however, because m^5C is a rare base in vertebrate DNAs (1 to 8% of total cytosines), photoproducts containing this base have yet to be identified and detected in a DNA sequence context. Ehrlich and Dove have studied the photochemistry at 254 nm of 5-methyldeoxycytidine (m^5dCyd) and detected at least 3 photoproduct peaks at a dose of 500 kJ/m^2 by TLC

(4). However, photolysis at 240 nm gave no indication of breakup of a putative $m^5dCyd-m^5Cyd$ photodimer despite irradiation of concentrated solutions.

The enzymatic methylation of cytosine by DNA (cytosine-5)-methyltransferase (DMase, EC 2.1.1.37) to form m^5C is both tissue and sequence specific (5,6). The biological importance of this reaction on gene regulation and possibly in chromosome organization is increasingly evident (7). A large body of evidence suggests an inverse correlation between the methylation of eukaryotic and viral genes and their expression which has been supported by the results of more recently microinjection and transfection experiments (8-10). The maintenance of a specific pattern of genomic methylation in differentiated cells by maintenance DMase activity appears necessary for phenotypic stability. Demethylating agents such as ethionine and 5-azacytidine which affect enzymatic methylation of the cell are known to produce significant effects on differentiation and gene expression (11,12). Similarly, UV radiation can lead to a specific loss of m^5C during subsequent rounds of replication or perhaps as a consequence of aberrant repair of the methylation pattern (13,14) and can produce transitions in the cell phenotype that lead to neoplastic transformation (15-17). The metallothionein gene, which is under putative methylation control as evidenced from 5-azacytidine reactivation experiments, can be derepressed from a quiescent hypermethylated state by UV irradiation (18). This suggests that infidelity in the repair of damaged tracts containing m^5C or the possible formation of photoproducts containing m^5C could have direct biological consequences. We have recently shown that far- and solar-UV-induced cyclobutyl pyrimidine photoproducts inhibit both maintenance and *de novo* methylation in a cell-free *in vitro* system (19). In order to detect possible m^5C photoproducts, highly purified rat liver DMase was used to methylate hemimethylated or unmethylated M13 double-stranded DNA and thereby specifically incorporate radioactively labeled methyl groups in m^5C in a DNA sequence context. We describe here the first detection of m^5C photoproducts in a DNA sequence context as a result of either solar or far-UV light.

MATERIAL AND METHODS

Specific labeling of m^5C in DNA

Single-stranded and replicative forms of M13 mp9 DNA were isolated as described in (20). Hemimethylated substrates were formed by primer extension of the phage DNA with dm^5CTP and large fragment DNA polymerase (19). These

were incubated with 1500-fold purified preparation of rat liver DMase that carries out both maintenance and de novo methylation functions (21) to generate specific methylation patterns in M13 substrates using S-adenosyl-L-(methyl-³H)-methionine (ICN, 9 Ci/mmol) at a concentration of 5 μ M for 6 h at 37°C and saturating amounts of DMase. Characterization of the specificity of the transmethylation of ³H-methyl groups to cytosine moieties was ascertained by HPLC analysis of the hydrofluoric acid hydrolysis (80°C, 1.5 h) (22) as shown in Figure 1A of the enzymatically methylated DNAs.

DNA Irradiation.

In vitro methylated DNAs were irradiated in the presence of molecular oxygen using either a Photodyne 3440A lamp (302 nm output in UVB) or Sylvania GT30TS, peak output at 254 nm as described by Becker et al. (19). Fluences were determined with Ultraviolet Products J221 and J220 calibrated UV light meters, respectively. HPLC-grade acetone at 10% v/v was employed as a triplet state photosensitizer of pyrimidine bases in the UVB range (23-24), and was removed prior to hydrolysis by lyophilization.

Photoproduct Determination

DNA samples containing photoproducts were hydrolyzed in concentrated formic acid (88%) for 35 min at 175°C (25). Resultant bases and photoproducts were separated by reverse phase HPLC on an Axiom (Cole-Parmer) 5 μ m C18 column (0.625 x 15 cm) programmed for elution with 100% buffer A (75 mM, potassium phosphate, pH 4.5) for 7 min, then a linear gradient to 60% buffer B (75 mM, potassium phosphate, pH 4.5, 40% methanol) for 30 min at 1 ml/min (19,26). Fractions were collected at 0.3 min intervals and the tritium label distribution and recovery was measured by liquid scintillation counting in Scintiverse II (Fisher Scientific). Using this program, elution times for authentic bases and photoproducts standards were: cytosine, 2.3 min; CT-cyclobutyl dimer, 3.5 min; uracil, 5.4 min; 5-methylcytosine, 7.6 min; guanine, 9.6 min; TT-cyclobutyl dimer, 11.2 min; 5-methyl-2'-deoxycytidine, 11.5 min; thymine, 12.5 min (19).

Nuclease P1 Protection and Endonuclease ν Assay for Photoproduct Formation.

Oligonucleotides and trinucleotides of defined sequence were synthesized using OCS Labs diisopropylphosphoramidite reagents (Denton, Texas) with an Applied Biosystems M 380A synthesizer. These sequences were confirmed by Maxam and Gilbert sequencing reactions (27), modified for small oligonucleotides (28). Oligonucleotide 5' termini were kinased with γ [³²P]-ATP (3000 Ci/mmol, Amersham) (27) and purified on silica-G thin layer chromatography plates. End-labeled trinucleotides were degraded to 5' mononucleotides with

P1 nuclease (Boehringer) (0.5 μ g for 1 h at 37°C in 20 mM PIPES, pH 6.0, 0.5 mM ZnCl_2) or to 3' mononucleotides with micrococcal nuclease (Worthington) (same conditions in 20 mM Tris-HCL pH 8.0, 0.5 mM CaCl_2), and directly loaded onto sequencing gels (24% polyacrylamide/8.3 M urea/90 mM Tris-borate, pH 8.3/1 mM EDTA) in gel loading buffer (90% formamide, 1 mM EDTA, 0.05% bromophenol blue and xylene cyanol). Specific formation of pyrimidine cyclobutane photodimers was assessed by susceptibility to cleavage by saturating amount of T4 endonuclease v (generously provided by Dr. N. Duker) which was purified from T4 infected *E. coli* (29). Irradiated oligonucleotides were treated with 1 μ g endonuclease v for 30 min at 37°C in 10 mM Tris-HCL pH 7.4/0.1 M NaCl/10 mM EDTA/100 μ g/ml nuclease free bovine serum albumin (19,30) and applied to sequencing gels and analyzed (31).

RESULTS AND DISCUSSION

HPLC Analysis

Figure 1A shows the radioactivity profile for de novo methylated M13 RF DNA using methyl groups transferred from the substrate [^3H -methyl]-AdoMet after hydrolysis with concentrated hydrofluoric acid (22). The single radioactive peak, containing over 98.5% of the radioactivity, co-eluted with authentic m^5C at fractions 21-23. Since the stability of pyrimidine photoproducts in hydrofluoric acid has not been systematically investigated, we chose to use the standard formic acid hydrolysis procedure that is well-characterized for the liberation of pyrimidine photoproducts (25). The hydrofluoric acid procedure does not produce an artifactual side product of m^5C hydrolysis which is shown in Figure 1B for the standard formic acid hydrolysis procedure applied to the same de novo methylated sample. The nature of the secondary peak in addition to authentic m^5C in Figures 1B, 1C, and 1D eluting at fractions 11-13 has been ascribed to a variable rate of conversion of m^5C by deamination to thymine (32). The chemical structure of this more polar formic acid hydrolysis product which has an absorbance maximum of 278 nm at pH 4.5 and elutes well before thymine (fractions 41-42) and 5-methyl-2'-deoxycytidine (fractions 38-39) on reverse phase chromatography is currently under investigation.

In preliminary experiments, irradiation with large doses of either UVC or UVB was employed to achieve sufficient yields of m^5C photoproducts for detection (Figures 1C, and 1D). After UV irradiation with a dose of 10 kJ/m^2 and formic acid hydrolysis to liberate bases and photoproducts, two additional small peaks could be detected and were identified as photoproducts I and II.

Figure 1. Reverse-phase separation of ^3H -methyl-labeled m^5C base and photo-products from *de novo* methylated M13 RF DNA using purified rat liver DNA methyltransferase. The distribution has been normalized to the total dpm recovered from each HPLC run. 1A; HPLC profile of hydrofluoric acid hydrolysis products of enzymatically methylated DNA; total dpm, 226,000. 1B; HPLC profile same as in Fig. 1A; hydrolysis conditions; concentrated formic acid (88%). 1C; UVC-irradiated, enzymatically methylated DNA (10 kJ/m^2 , 254 nm); hydrolyzed with formic acid, same as in Fig. 1B; total dpm, 99,600; 1D; UVB-irradiated, enzymatically methylated DNA (10 kJ/m^2 , 302 nm); hydrolysis conditions, same as in Fig. 1B; total dpm, 109,100. Photoproducts I and II designated by stippled and dashed areas eluted at 3 and 6.7 min, respectively.

The m^5C containing photoproduct I eluted at fractions 7-9 and represents the conversion of 5.5% of the radiolabeled m^5C precursor at 254 nm and 12% at 302 nm. Photoproduct II, which eluted just ahead of m^5C at fractions 14-16, comprised 3.8% at 254 nm (Figure 1C) and 4.8% at 302 nm (Figure 1D). Irradiation of DNA in the absence of photosensitizer in UVB or UVC range can be used to change the ratio of thymine-containing to cytosine-containing photodimers

Figure 2. Effect of triplet-state sensitizer on the yield of UVB-induced m^5C -containing photoproducts. 2A; UVB-irradiated (3.6 kJ/m^2 , 302 nm) in the absence of acetone; total dpm, 89,800. 2B; UVB-irradiated (as above) in the presence of 10% acetone; total dpm, 84,300; DNA substrate, HPLC and hydrolysis conditions, same as in Fig. 1B.

in favor of the production of the latter with UVB (33). This effect is related to the overlap in the 302 nm (UVB) wavelength range with the absorbance maximum of cytosine (280 nm) and, thus, the excitation of m^5C should be similar to that of cytosine rather than thymine as the m^5C absorbance maximum occurs at 282 nm which furthermore results in the excitation of a m^5C fluorophore at physiological pH values (3).

The formation of pyrimidine cyclobutyl dimers is thought to proceed mainly via excitation to a triplet state, whereas monomeric peroxides and hydrates are formed from singlet state transitions (34). The yield of cyclobutyl dimers can be enhanced by energy transfer between a photosensitizer molecule with excitation energy (E_t) greater than that of the energy of the triplet state of the base to be sensitized. Acetone, which has an E_t of $28,200 \text{ cm}^{-1}$ which is greater than the canonical bases, enhances the yield of both cytosine- and thymine-containing cyclobutyl dimers in the UVB portion of the spectrum (19,23,24,34) and thus should photosensitize m^5C . In Figure 2, de novo enzymatically methylated M13 RF DNA was irradiated with a dose of 3.6 kJ/m^2 at 302 nm in either the presence or absence of acetone. At this lower dose, in the absence of acetone, the yield of photoproduct II was imperceptible over the background dpm in the region between fractions 19-21

(Figure 2A). In the presence of acetone, the same UVB dose enhanced the formation of photoproduct II (Figure 2B). The yield and formation of photoproduct I was not significantly changed, suggesting that the conversion of m^5C to photoproduct I goes through a singlet manifold. Since this compound eluted much earlier than m^5C , it is more polar; and, from its stability in solution, may be a photohydrate rather than a glycol or peroxide. Photoproduct II formation is enhanced by the presence of triplet-state sensitizer and, therefore, is likely to represent a dimeric m^5C -containing cyclobutyl type of compound.

These suppositions about the structure of the purified photoproducts were further investigated by direct photolysis to investigate the reversal of putative m^5C photoproducts (Figure 3). Large amounts of tritiated methyl labeled m^5C in a DNA sequence context were generated by a maintenance methylase-catalyzed reaction with purified rat liver DMase on hemimethylated substrate (second strand synthesis of viral M13 DNA in the presence of dm^5CTP instead of $dCTP$) (19,21). After hydrolysis, photoproducts I and II were then purified by HPLC as in Figure 2B and yielded as shown in Figures 3A and 3B, a single peak upon rechromatography comprising approximately 99% and 96.5% of the input counts, respectively. Purified photoproduct I, when photolysed with 10 kJ/m^2 254 nm, was partially converted to m^5C and the formic acid- m^5C derivative: yield 42% and 10%, respectively, with 45% photoproduct I remaining (Figure 3C). Photoreversion of photoproduct I did not lead to any detectable formation of a photoproduct II peak. Photoreversion of photoproduct II yielded a mixture of 27% photoproduct I, 45% m^5C and 15% of the formic acid- m^5C derivative with 7% photoproduct II remaining (Figure 3D). The formation of m^5C -containing photoproducts I and II in DNA is clearly demonstrated from the regeneration of precursor m^5C on photolysis. These data support the idea that photoproduct II is dimeric as photoproduct I was not converted to photoproduct II by photolysis whereas reirradiation of photoproduct II gave rise to the monomeric product. The elution position of photoproduct II suggests photodimerization with cytosine, e.g., the elution position of CT- and TT-cyclobutyl dimers, when separated by reverse phase chromatography using the conditions in Figure 1 occurred slightly before the elution position of the trailing base (19,26). In this case, a Cm^5C -cyclobutyl dimer should elute just before m^5C as was observed for photoproduct II, whereas, a Tm^5C cyclobutyl dimer should elute on this HPLC program between 7.6 and 12.5 min, the respective elution times of m^5C and thymine.

Figure 3. Photolysis products of purified m^5C -containing photoproducts I and II. Photoproducts I and II were isolated as in Fig. 1 from formic acid hydrolyzate of 5 μ g hemimethylated M13 DNA enzymatically methylated to a specific activity of 9.5×10^5 dpm/ μ g ^3H -methyl incorporated in m^5C . The isolated photoproducts were then reirradiated with a dose of 10 kJ/m² 254 nm light and the photolysis products determined by isocratic elution with buffer A. 3A; HPLC profile of isolated photoproduct I before photolysis; total dpm 10,380. Peak denoted by dotted fractions contain approximately 99% of input dpm. 3B; HPLC profile of isolated photoproduct II before photolysis; total dpm 4,840. Peak denoted by stippled fractions containing approximately 96.5% of input dpm. 3C; HPLC profile of photolyzed photoproduct I; total dpm, 52,500. 3D; HPLC profile of photolyzed photoproduct II; total dpm, 25,900.

Enzymatic Analysis of Photochemically Altered m^5C in Oligonucleotides

To provide further evidence that m^5C can participate in photoproduct formation in a DNA sequence context, d[TTG] and d[Cm⁵CG] trinucleotides were synthesized and irradiated with 10 kJ/m² 254 nm or 302 nm ultraviolet light, respectively. Irradiation of d[TTG] at 254 nm was used to generate TT cyclo-

Figure 4. Polyacrylamide gel electrophoretic analysis of trinucleotides subjected to UV irradiation 10 kJ/m^2 . Panel A, $d[\text{Cm}^5\text{CG}]$; Panel B, $d[\text{TTG}]$. Lanes 1 show untreated control before irradiation; lanes 2 after 10 kJ/m^2 254 nm for $d[\text{TTG}]$ or 302 nm for $d[\text{Cm}^5\text{CG}]$; lanes 3 same as lane 1 after micrococcal nuclease treatment; lanes 4 same as lane 2 after micrococcal nuclease treatment; lanes 5 same as lane 1 after nuclease P1 treatment; lanes 6 same as lane 2 after nuclease P1 treatment.

butyl dimers that could be used as a standard to compare the effects of 302 nm on the $m^5\text{C}$ -containing trinucleotides. Both unirradiated and irradiated samples were then degraded with micrococcal nuclease or nuclease P1 and the resultant mixture analyzed on 24% sequencing gels (Figure 4). As shown in lane 2, after irradiation a minor component of $d[\text{TTG}]$ and $d[\text{Cm}^5\text{CG}]$ migrates with a slower electrophoretic mobility than the unirradiated trinucleotides shown in lane 1. After digestion with either micrococcal nuclease (lane 3) or nuclease P1 (lane 5) the parental trinucleotides are completely degraded yielding 5' PdNP and 5' dNMP, respectively, as well as traces of undegraded end-labeled dinucleotides. However, similar treatment with these nucleases does not degrade the altered mobility component arising from irradiation (lanes 4 and 6), indicating that a photochemical reaction has taken place. These results suggest that adjacent Cm^5C pyrimidines in DNA behave in a similar fashion as adjacent TT pyrimidines after irradiation with appropriate wavelength ultraviolet light. The resistance of the irradiated trinucleotides to enzymatic cleavage is consistent with a photodimerization event holding the nucleotides together via a cyclobutane ring, or rendering the trinucleotides resistant to degradation by photoproduct alteration of the substrate, or both.

Endonuclease v, a UV-specific endonuclease encoded by the T4 *denv* gene,

Figure 5. Analysis of photoproduct formation by site specific cleavage with T4 endonuclease v. 5'end-labeled control dodecanucleotide (lanes 1-8, sequence shown in lanes 5-8) and its m^5C containing homolog (lanes 9-18, sequence in lanes 9-12) were analysed for distribution of photoproducts after irradiation with 10kJ/m^2 302 nm light. Lane 1, irradiated control oligo; lane 2, same as lane 1 but treated with endo v as in Materials and Methods; lane 3, unirradiated oligo; lane 4, same as lane 3 treated with endo v; lane 5, oligo sequenced by purine specific reaction with formic acid, lane 6, oligo sequenced by pyrimidine specific reaction with hydrazine, lane 7, oligo sequenced by cytosine specific reaction with hydroxylamine, lane 8, oligo sequenced by thymine specific reaction using KMnO_4 . Center lane shows the deduced sequence of the oligo and m^5C -containing oligo (Note sequencing lanes are vastly overexposed compared to endo v treated lanes in order to detect the very small amounts of enzymatic cleavage in the latter). Lane 9, m^5C -oligo same as lane 5; lane 10, m^5C -oligo same as lane 6; lane 11, m^5C -oligo same as lane 7; lane 12, m^5C -oligo same as lane 8; lane 13, unirradiated m^5C -oligo; lane 14, same as lane 13 treated with endo v; lane 15, irradiated m^5C -oligo; lane 16, irradiated m^5C -oligo treated with endo v (single-stranded substrate); lane 17, same as lane 15 m^5C -oligo annealed to M13 phage DNA (double-stranded substrate); lane 18, same as lane 17 treated with endo v; BPB, bromophenol blue migration position (artificial bands).

preferentially recognizes pyrimidine photodimers primarily in double-stranded, but also in single-stranded DNA (31). Since it has been shown to recognize purine-pyrimidine photoproduct lesions in DNA (30), its broad spectrum of activity against UV photoproducts suggests it might be useful for detecting

m^5C -containing photoproducts in DNA. We initially explored this possibility in the trinucleotide where the photochemically altered mobility/nuclease PI-resistant component (Figure 4) could be isolated and tested for specific cleavage after alkali treatment if an apyrimidinic site were generated by the intrinsic DNA-glycosylase activity in *endo v* (31). These results were negative (data not shown) and likely due to the size of the trimers. Longer m^5C -containing oligonucleotides that could be annealed to DNA were therefore synthesized in order to explore specific *endo v* cleavage at photochemically altered m^5C sites. In Figure 5, an example is shown of a pair of dodecanucleotides (sequence 5' d[GGTCGACGGATCCCCGGGAA] 3') having, in one case, a replacement of m^5C in the *SalI* d[GT m^5C GAC] and *SmaI* d[CC m^5C GGG] sites. The position of the m^5C was confirmed from the m^5C induced inhibition of *SalI* and *SmaI* cleavage (gel not shown), and from direct sequence determination using hydrazine, hydroxylamine, and $KMnO_4$ reactivities that recognize: C+T; C; strong T/weak m^5C , respectively (27,28). The deduced sequence is shown in the center lanes of Figure 5. When these dodecamers were irradiated with 10 kJ/m² 302 nm light, no mobility shift could be resolved; however, a clear indication of cytosine photodimers can be detected in the run of cytosines in the *SmaI* site after incubation with *endo v* (lane 2). Similarly, in the UVB-irradiated m^5C -oligonucleotide both cytosines and m^5C in the *SmaI* site are recognized by the *endo v* endonucleolytic activity specific for the apyrimidinic site generated by the *endo v* glycosylase activity. This creates molecules having sizes of the corresponding sequencing reaction products, however, with a slightly altered mobility in sequencing gels if treated with alkali to remove the 3' apyrimidinic deoxyribose (31). This photochemical event is clearly shown in lanes 16 and 18 for single- and double-stranded substrate forms, respectively, where in each case there is a fragment arising from cleavage between the m^5C and 3' adjacent cytosine in the *SmaI* site. Interestingly, the m^5C and cytosine in the *SalI* site having a thymine on the 5' side did not show significant *endo v* cleavage after 10 kJ/m² 302 nm light. This observation may be related to the known enhancement of photodimerization events in polypyrimidine stretches as compared to single pyrimidine pairs (35) and the low absorbance of thymine at this wavelength (1,34) that would adversely affect the yield of a thymine-containing photoproduct.

The above results indicate that both solar and far-UV can produce photochemical alterations in m^5C in DNA. At least two photoproducts were detected by following specifically *in vitro* labeled m^5C moieties in DNA by

HPLC. Two peaks could be routinely detected after irradiation at 10 kJ/m^2 . These peaks, when isolated, photolysed, and rechromatographed, regenerated the labeled m^5C precursor, indicating that the designated photoproducts I and II are composed of authentic m^5C ; however, it is not clear whether each is composed of a single species until chemical structure determinations are performed. Evidence that photoproduct II is dimeric could be deduced from its breakup, which generated photoproduct I and its elution position that suggested a photodimer with cytosine or possibly m^5C . The likelihood for the occurrence of the latter m^5C - m^5C photodimer is highly improbable both herein and in vivo since the specificity of the DNA methyltransferase gives very little indication of any outer C methylation in d[CCGG] sites that would inhibit MspI cleavage. Since photoproduct I did not form photoproduct II after photolysis, it appears to be a monomeric species. m^5C photoproducts are calculated to be formed in the low ppb range in vertebrate DNA when irradiated with biologically relevant doses ($<300 \text{ J/m}^2$). Elucidation of the possible chemical structure of these photoproducts is currently under investigation using mass spectrographic methods due to the minute quantities that can be isolated. Because of this, current methods to investigate their presence in vivo or in particular sequences as shown for synthetic m^5C -containing oligonucleotides in Figures 4 and 5 will depend on scaled up procedures for isolation for chemical structure determination and to develop radioimmuno-logical methods that are currently available to detect TT cyclobutyl photodimers in DNA (36).

Acknowledgements.

We acknowledge the thoughtful reading of this manuscript by Kathleen Robertson and wish to thank Betty Martz for help in preparation of the manuscript.

This investigation was supported by U.S. Public Health Service grants CA31487 awarded by the National Cancer Institute, and by a fellowship in toxicology awarded to M.R. by Conoco, Inc.

Abbreviations used are: DMase, DNA (cytosine-5)-methyltransferase (EC 2.1.1.37); endo v, endonuclease v (T4 UV endonuclease) coded by the denV gene of bacteriophage T4 from T4-infected *E. coli*; HPLC, high performance liquid chromatography; oligo, oligodeoxyribonucleotide; UVB, portion of ultraviolet spectrum from 280-340 nm.

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