# A Cytosine Methyltransferase Converts 5-Methylcytosine in DNA to Thymine†

María J. Yebra‡ and Ashok S. Bhagwat*

*Department of Chemistry, Wayne State University, Detroit, Michigan 48202-3489*

*Received July 21, 1995; Revised Manuscript Received September 15, 1995*⊗

ABSTRACT: Sites of cytosine methylation are known to be hot spots for C·G to T·A mutations in a number of systems, including human cells. Traditionally, spontaneous hydrolytic deamination of 5-methylcytosine to thymine has been invoked as the cause of this phenomenon. We show here that a bacterial cytosine methyltransferase can convert 5-methylcytosine in DNA to thymine and that this reaction creates a mutational hot spot at a site of DNA methylation. The reaction is fairly insensitive to the methyl donor in the reaction, *S*-adenosylmethionine. In many cancers, the most frequent class of mutations is C to T changes within CG dinucleotides of the tumor suppressor gene p53. Because of the similarities of the reaction mechanisms of mammalian and bacterial enzymes and the physiology of the cancer cells, this reaction is expected to contribute to mutations at CG dinucleotides in precancerous cells.

Study of spontaneous mutations in bacterial genes (Coulondre et al., 1978; Lieb, 1991) and of mutations that cause human diseases (Brennan et al., 1990; Cooper & Youssoufian, 1988; Giannelli et al., 1990; Hirschhorn et al., 1990; Koeberl et al., 1990; Perutz, 1990) has revealed that sites of cytosine methylation in DNA are hot spots for C to T changes. For example, there is a striking correlation between some types of cancers and mutations at CG dinucleotides in the tumor suppressor gene p53 (Greenblatt et al., 1994; Jones et al., 1992; Laird & Jaenisch, 1994). It has been suggested (Coulondre et al., 1978; Lindahl & Nyberg, 1974) that such mutations result from spontaneous hydrolytic deamination of 5-methylcytosine in DNA to thymine.

Methyltransferases transfer methyl groups to position 5 of cytosine (C5 MTases) by an addition−elimination mechanism (Santi et al., 1983). The first step in the reaction involves an attack at C6 of cytosine by cysteine thiolate (Chen et al., 1991; Friedman & Ansari, 1992). Because cytosine derivatives with a saturated 5,6 bond are thought to be $10^4$ times more susceptible to hydrolytic deamination than cytosine (Shapiro, 1981), Selker (1990) suggested that under certain conditions C5 MTases may convert cytosine to uracil or to thymine during a methyl transfer reaction. In effect, the action of the enzymes would resemble the action of bisulfite which adds across the 5,6 double bond of cytosine and promotes the deamination of this base (Hayatsu, 1976).

Recently, one part of this prediction was confirmed for several C5 MTases. A genetic reversion assay was used to show that bacterial MTases M.*Hpa*II (Bandaru et al., 1995; Shen et al., 1992), M.*Eco*RII (Wyszynski et al., 1994), M.*Sss*I (Bandaru et al., 1995), and M.*Hha*I (Yang et al., 1995) can convert cytosine in DNA to uracil when the methyl donor *S*-adenosylmethionine (SAM) is omitted from an *in vitro* reaction. However, SAM was found to be a strong inhibitor of this reaction (Shen et al., 1992; Wyszynski et al., 1994), and no revertants were detected when physiological concentrations of SAM were included in the reaction. Further, both *in vitro* (Shen et al., 1992) and *in vivo* (Bandaru et al., 1995; Shen et al., 1992; Wyszynski et al., 1994) uracil-DNA glycosylase (UDG) was found to efficiently remove the uracil generated by C5 MTases and suppress the mutations. In addition, DNA mismatch correction processes that are thought to be designed to repair T·G mismatches also repair U·G mismatches to C·G (Gabbara et al., 1994; Neddermann & Jiricny, 1994). These factors present significant barriers against C to U conversions contributing to mutations at sites of cytosine methylation.

We reasoned that, if C5 MTases could convert 5-methylcytosine (5meC) to thymine, this would have a greater likelihood of contributing to C to T mutations. In part, this is because DNA repair processes that are known to act on T·G mismatches within sites of methylation are known to be inefficient (Bandaru et al., 1995; Brown & Jiricny, 1987; Lieb, 1991). C5 MTases could convert 5meC to T by a mechanism similar to that by which they convert C to U. The reaction would involve the formation of a covalent adduct between the enzyme and the methylated DNA followed by protonation of the unstable carbanion (Figure 1). This reaction is feasible because the attack of C6 by the enzyme does not require SAM (Gabbara et al., 1995; Wu & Santi, 1987). The resulting dihydrocytosine adduct should be more susceptible to hydrolytic deamination than 5meC. Thus, although an enzyme·SAM or an enzyme·SAH (SAH = *S*-adenosylhomocysteine) complex is not expected to reinitiate the catalytic cycle, there is no reason why the enzyme by itself could not initiate such a reaction. We tested this prediction by studying the ability of a bacterial C5 MTase to cause C to T mutations and show here that this MTase can effect such conversions.

## MATERIALS AND METHODS

*Purification of EcoRII MTase and Plasmid DNA.* The M.*Eco*RII used here was purified using a modification of the procedure described previously (Gabbara et al., 1995). Briefly, the gene for the MTase was expressed from a phage T7 promoter, and the cells were disrupted by sonication.

† This work was supported by NIH Grant GM45860 (to M. Lieb, USC Medical School, and A.S.B.) and by Research Career Development Award HG00004 to A.S.B.

* Corresponding author. Telephone: 313-577-2547. Fax: 313-577-1377. E-mail: axb@chem.wayne.edu.

‡ Present address: Instituto de Agroquimica y Tecnologia de Alimentos, CSIC, Jaime Roig 11, E-46010 Valencia, Spain.

⊗ Abstract published in *Advance ACS Abstracts,* November 1, 1995.

0006-2960/95/0434-14752$09.00/0 

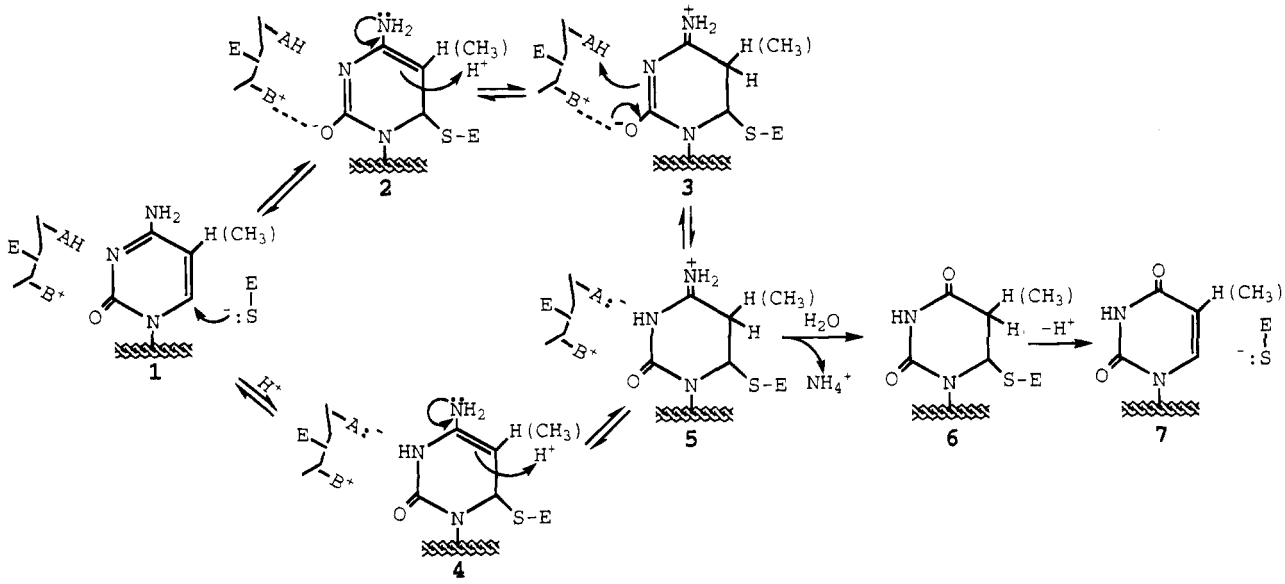

FIGURE 1: Mechanism of enzyme-mediated hydrolytic deamination of cytosine and 5-methylcytosine. The mechanism is based on the current understanding of the mechanism of methyl transfer by C5 MTases (Bestor & Verdine, 1994; Gabbara et al., 1995; Smith, 1994) and known susceptibility of cytosine derivatives with substitutions at N3 to deamination (Sowers et al., 1989). The target cytosine (5-methylcytosine) is thought to be flipped out of the double helix, and C6 of the base is attacked by a cysteine thiolate in the enzyme. Subsequent charge delocalization is expected to create two possible enamine-containing intermediates, **2** and **4**. Protonation of these intermediates should result in the formation a positively charged iminium species **5**. The iminium ion should readily undergo hydrolysis, creating uracil (thymine).

Previously, lysozyme treatment was used to break the cells. Omitting this step from the purification procedure prevented the contamination of the enzyme with lysozyme in early steps of purification and reduced the number of columns needed for purification to two-phosphocellulose and DEAE sephacel. The resulting preparation was judged to be homogeneous by gel electrophoresis. Plasmid DNA carrying the mutated *kan* gene (pKanS-D94) was isolated by alkaline lysis method followed by a CsCl gradient and dialysis against TE (Tris-HCl, pH 7.8, 1 mM EDTA).

*Incubation of DNA with M.EcoRII and the Reversion Assay.* The conditions used in experiments where unmethylated pKanS-D94 DNA was incubated with M.*Eco*RII have been described before (Wyszynski et al., 1994). In this case, the treated DNA was electroporated into GM31 *ung* (*dcm-6 ung-1 tyrA*::Tn*10 thr-1 hisG4 leuB6 rpsL ara-14 supE44 lacY1 tonA31 tsx-78 galK2 galE2 xyl-5 thi-1 mtl-1*), an *ung*$^-$ strain that carries a defect in the chromosomally coded C5 MTase Dcm and in the specialized mismatch correction process, very short patch repair (Dar and Bhagwat, 1993; Wyszynski et al., 1994). Transformants were plated onto LB plates with carbenicillin (50 $\mu$g/mL) or kanamycin (50 $\mu$g/mL). Reversion frequency was calculated as the ratio between the numbers of kanamycin-resistant transformants and carbenicillin-resistant transformants. Relative reversion was defined as the ratio of reversion frequency resulting from incubation of DNA with M.*Eco*RII and resulting from incubation of DNA without the enzyme for the same length of time.

The conditions for complete methylation of pKanS-D94 were determined by using [*methyl*-$^3$H] SAM in the reaction and by monitoring the incorporation of the radiolabel in DNA. Several micrograms of pKanS-D94 were methylated with a 5-fold molar excess of M.*Eco*RII and 50 $\mu$M SAM, in 1× MTase buffer [100 mM Tris-HCl (pH 7.8), 20 mM EDTA (pH 8.0), 0.4 mM dithiothreitol]. The reaction was carried out at 37 °C for 30 min. The protein was removed with phenol−chloroform extraction, and SAM was removed by gel filtration with Sephadex G50. In the second step, 1−2 $\mu$g of the methylated plasmid was incubated with a 5-fold molar excess of M.*Eco*RII in 1× MTase buffer. In some cases, different concentrations of SAM or SAH were also included in the reactions. Incubations were carried out at 37 °C for various lengths of time, and the reactions were stopped by phenol−chloroform extractions. Similar reactions were also carried out in the absence of the MTase. In both cases, the DNA was phenol−chloroform extracted, filtered over a G-50 column, dried down, and resuspended in 5 $\mu$L of TE buffer [10 mM Tris-HCl (pH 7.8), 0.1 mM EDTA]. It was electroporated using the Cell-Porator E. coli pulser (GIBCO BRL) into GM31, the *ung*$^+$ parent of the strain mentioned above, and reversion frequencies were determined.

*Treatment of DNA with UDG.* In one set of experiments following the second treatment of plasmid DNA with M.*Eco*RII, the DNA was further treated with uracil DNA glycosylase (UDG, GIBCO BRL) before electroporating it into GM31. The reaction was carried out in 1× UDG buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl, 5 mM $MgCl_2$), with 2 units of enzyme, for 1 h at 37 °C. The enzyme was removed by phenol−chloroform extraction, and the Sephadex G-50 columns were used to remove phenol−chloroform prior to the electroporation of DNA. The reversion frequencies were calculated as described above.

## RESULTS

We tested the ability of an *Escherichia coli* C5 MTase, M.*Eco*RII, to carry out a 5meC to T conversion reaction using a reversion assay involving kanamycin resistance (Wyszynski et al., 1994; and Figure 2). Previously methylated plasmid DNA carrying the *kan* allele was incubated with fresh M.*Eco*RII for various lengths of time, the DNA was electroporated into cells containing UDG (*ung*$^+$) cells,

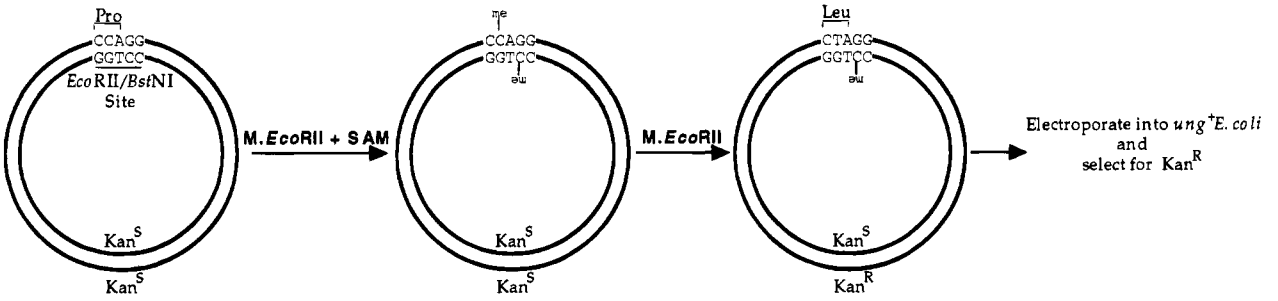


FIGURE 2: Scheme of an experiment to test 5MeC to T conversion by M.*Eco*RII. The test plasmid, pKanS-94D, carries an inactive allele of the *kan* gene from Tn*5*. It contains a Leu to Pro change at codon 94, resulting in a new site for methylation by the *E. coli* C5 MTase, M.*Eco*RII. Conversion of the second C in the M.*Eco*RII recognition sequence (CCAGG) to T restores a Leu codon and kanamycin resistance (Wyszynski et al., 1994).

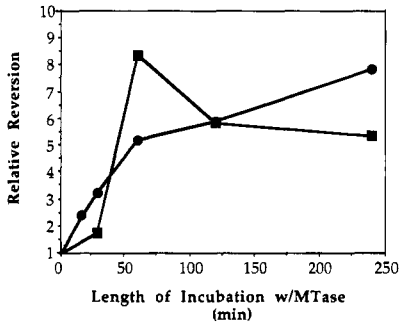


FIGURE 3: Conversion of 5-MeC to T by M.*Eco*RII. Previously methylated pKanS-94D DNA was incubated with M.*Eco*RII for various lengths of time and electroporated into the *ung*$^+$ *E. coli* strain GM31, and kanamycin-resistant transformants were scored (■). In separate reactions, the MTase-treated DNA was further incubated with UDG and electroporated into the same *E. coli* strain (●). The reversion frequencies of MTase-treated DNAs relative to those of the untreated DNA are reported.

Table 1: Efficiencies of C to U and 5meC to T Conversions

| methylation[a] status of DNA | reversion frequency: without MTase | reversion frequency: with MTase | relative reversion[b] | rate ($s^{-1}$)[c] |
|---|---|---|---|---|
| methylated | $3.9 \times 10^{-8}$ | $(1.7 \pm 1.9) \times 10^{-7}$ | 4.3 | $3.6 \times 10^{-10}$ |
| unmethylated | $1.9 \times 10^{-8}$ | $(5.7 \pm 4.3) \times 10^{-6}$ | 293 | $1.6 \times 10^{-8}$ |

[a] Following incubation with the MTase in the absence of cofactor, the DNA was transformed into *ung*$^-$ (unmethylated DNA) or *ung*$^+$ (methylated DNA) to score for kanamycin-resistant revertants. [b] Ratio of reversion frequency with and without the MTase. [c] Calculated as the rate of increase in the reversion frequency per second of incubation with the MTase.

and kanamycin-resistant (Kan$^R$) transformants were scored. Incubation of DNA with the enzyme resulted in a time-dependent increase in reversions to Kan$^R$ (Figure 3). Although some variation was seen from experiment to experiment (Figure 3 and Table 1), a 1 h incubation with the enzyme caused a 4−10-fold increase in the reversion frequency. The nature of the mutation in the revertants was determined by digesting plasmid DNA from three independent revertants with restriction enzyme *Bst*NI. As expected, all three plasmids had lost the diagnostic *Bst*NI site at codon 94 of *kanS* (not shown).

It is unlikely that revertants came about due to a C to U conversion by the enzyme followed by the replication of DNA. As the DNA was methylated with M.*Eco*RII in the first step, few, if any, molecules are expected to be available for C to U conversion. Further, we (Wyszynski et al., 1994) and others (Shen et al., 1992) have shown that, when *ung*$^+$ *E. coli* is used as the recipient in electroporation, the increase in Kan$^R$ revertants due to MTase-mediated C to U conversion is completely eliminated. To further assure that the revertants did not arise by the C to U to T pathway, we carried out experiments in which MTase-treated DNA was incubated with UDG *in vitro* prior to its electroporation into *ung*$^+$ *E. coli*. As expected, it was found that the incubation of DNA with UDG had little effect on the frequency of Kan$^R$ revertants (Figure 3). We confirmed that UDG used in the experiments was enzymatically active by testing its ability to suppress C to U conversions promoted by M.*Eco*RII. Unmethylated DNA was first incubated with M.*Eco*RII to cause C to U conversions. The reaction mixture was divided into two halves, and one half was electroporated into *ung*$^-$ cells to quantitate the extent of C to U conversions. The other half was treated with UDG prior to its electroporation in the same *E. coli* host. It was found that the UDG treatment reduced the Kan$^R$ reversion frequency by a factor of 183 (not shown).

Because M.*Eco*RII shows lower affinity toward methylated DNA compared to unmethylated DNA (Wyszynski et al., 1993), the enzyme would be expected to cause C to U conversions at a higher rate than that for 5meC to T conversions. This was confirmed by carrying out parallel incubations of unmethylated or methylated DNAs with M.*Eco*RII, followed by transformation of the DNAs in appropriate strains (Table 1). Because of the differing levels of background reversion frequencies for the two types of DNA, the reversion frequencies obtained in the two reactions could not be directly compared to each other. Instead, we compared the rates of the two reactions and found that the rate of C to U conversions was about 44-fold higher than that for the 5meC to T conversion (Table 1). Despite this, the rate of enzyme-promoted 5meC to T conversion was about 620 times higher than the known rate of spontaneous hydrolytic deamination of 5meC in double-stranded DNA (Shen et al., 1994).

The C to U conversion reaction promoted by the C5 MTases is very sensitive to presence of SAM in the reaction. Such conversions are completely inhibited at concentrations of SAM above 0.8 $\mu$M (M.*Hpa*II; Shen et al., 1992) or 0.63 $\mu$M (M.*Eco*RII; Wyszynski et al., 1994). Similar concentrations of SAH also inhibit these reactions (Shen et al., 1992; Wyszynski et al., 1994). The concentration of SAM in different animal tissues is found to vary in the 20−70 $\mu$M range (Eloranta, 1977; Hibasami et al., 1980), while the concentration of SAH ranges between 2 and 40 $\mu$M (Eloranta, 1977). This is one of the reasons why there is scepticism (Lindahl, 1993; Smith, 1994; Wyszynski et al.,

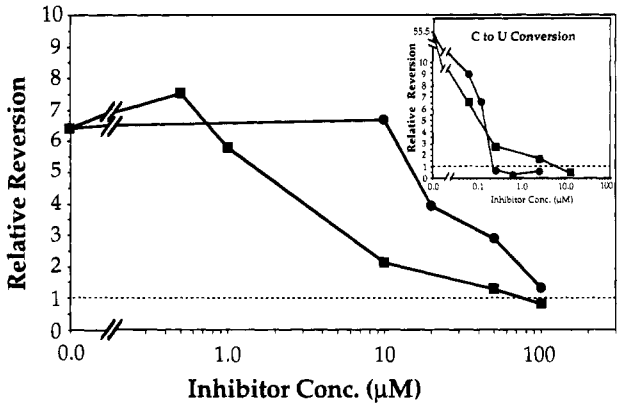


FIGURE 4: Inhibition of 5meC to T conversion by SAM and SAH. Various concentrations of SAM (●) or SAH (■) were tested for their ability to inhibit conversion of 5meC to T. In all cases, the incubations were carried out with a 5-fold molar excess of the enzyme for 60 min. The inset contains data on the inhibition of C to U conversion by M.*Eco*RII. Unmethylated pKanS-94D was incubated with a 3.5-fold excess of M.*Eco*RII in the presence of various concentrations of SAM (●) or SAH (■). The C to U conversion data have been previously reported (Wyszynski et al., 1994).

1994) about whether this reaction could contribute to mutations at sites of DNA methylation in human tissue.

We found that the 5meC to T conversion reaction was substantially less susceptible to inhibition by SAM (Figure 4). Unlike the C to U conversion reaction, 5meC to T conversion by the enzyme could be detected at concentrations of SAM as high as 50 $\mu$M (Figure 4). This is ~100 times higher than the $K_m$ of M.*Eco*RII for SAM in the methyl transfer reaction (0.47 $\mu$M; Friedman, 1985). We attribute the relative lack of sensitivity of the reaction to SAM to the presence of the methyl groups in cytosines. Presumably, SAM cannot enter the enzyme·DNA complex because of steric hindrance between the methyl group in SAM and that in 5meC. Consistent with this hypothesis, the reaction was found to be somewhat more sensitive to SAH. But even with SAH, concentrations of the cofactor of up to 10 $\mu$M were unable to eliminate M.*Eco*RII-mediated 5meC to T conversions (Figure 4).

Although we have demonstrated 5meC to T reaction with a bacterial enzyme, there is every reason to believe that the eukaryotic MTases will also be able to carry it out. The reaction mechanism of the human enzyme is known to be similar to its bacterial counterparts (Smith et al., 1992). In fact, the mammalian MTases may be able to carry out significant amounts of 5meC to T conversion at normal physiological concentrations of SAM and SAH. This is because the reported $K_m$ of mammalian C5 MTases for SAM is about 5-fold higher than that of M.*Eco*RII (Simon et al., 1978).

## DISCUSSION

*C5 MTase Levels, DNA Methylation, and Cancer.* There is an interesting correlation between DNA methylation and carcinogenesis. As much as a 3000-fold overexpression of cytosine methyltransferase activity is found in some tumorigenic cell lines (Kautiainen & Jones, 1986), and increases in the level of MTase gene expression are found to correlate well with progression of colon cancer (El-Deiry et al., 1991). The overexpression of MTase during neoplastic transformation may be the result of the regulation of its gene by AP-1 and Ha-Ras at the level of transcription (Rouleau et al., 1995). Additional evidence for the correlation comes from experiments in which increasing the amount of MTase activity in NIH3T3 cells caused phenotypic changes that are characteristic of neoplastic transformation and increased the tumorigenicity of the cells (Wu et al., 1993). Finally, reduction of DNA methyltransferase activity in the *Min* strain of mouse substantially suppresses the occurrence of intestinal adenomas (Laird et al., 1995).

Paradoxically, this increase in MTase activity is accompanied by a decrease in the amount of 5-methylcytosine in neoplastic tumors (Feinberg et al., 1988; Goelz et al., 1985). Together these studies suggest a view of precancer cells in which there is relative abundance of MTase, but where the enzyme carries out little additional methylation. Although the excess MTase in the cells could promote carcinogenesis by changing the pattern of methylation of specific genes (Jones et al., 1992), it is attractive to consider the possibility that it may accelerate the process of neoplastic transformation by causing mutations.

*Relationship of SAM Levels in Cells to Cancer.* The paradox between higher levels of MTase activity and hypomethylation would be resolved if tumor (or pretumor) cells were to contain lower levels of SAM than normal cells (Laird & Jaenisch, 1994). In a series of studies, Feo and his colleagues (Feo et al., 1985; Garcea et al., 1987; Pascale et al., 1992; Simile et al., 1994) investigated the relationship between carcinogen-induced liver cancer and SAM levels and found that progression of disease correlated with reductions in SAM levels. Rats were subjected to a complex regimen involving treatment with diethylnitrosamine and phenobarbitol followed by partial hepatectomy and further treatment with 2-acetylaminofluorene. Following this treatment, SAM levels in the liver decreased to 53−61% of their original value (Feo et al., 1985) and were 39% of their original value in the visible hyperplastic nodules (Garcea et al., 1987). This was accompanied by hypomethylation of DNA and development of neoplasias (Garcea et al., 1987; Simile et al., 1994). Remarkably, treatment of these rats with SAM resulted in a decrease in the number and size of the nodules and other markers for neoplastic development (Pascale et al., 1992).

Other types of studies also support a relationship between SAM levels and cancer. Rats on diets restricted in methyl-supplying lipotropes methionine, choline, folic acid, and vitamin $B_{12}$ (Christman et al., 1993; Henning et al., 1989; Shivapurkar & Poirer, 1983) or suffering from vitamin $B_{12}$ deficiency (Doi et al., 1989) have lower levels of SAM. Further, methyl donor-restricted diets also cause hypomethylation of DNA (Christman et al., 1993; Dizik et al., 1991; Wainfan & Poirier, 1992) and tumorigenesis in mice and rats (Newberne & Rogers, 1986; Poirer et al., 1986; Yokoyama et al., 1985).

*Cofactor Levels and Mutations in Precancer Cells: A Hypothesis.* We have shown here that a likely effect of lowering SAM levels in precancer cells is an increase in MTase-mediated 5meC to T conversions. Earlier, Jones and his colleagues (Shen et al., 1992) and we (Wyszynski et al., 1994) demonstrated a similar effect regarding MTase-mediated C to U conversions. Because the enzyme-mediated 5meC to T conversion is less susceptible to SAM or SAH than the C to U conversion (Figure 4), even if the decrease

in SAM and SAH levels in precancer benign tumors is small, this could still result in a substantial increase in 5meC to T conversions. In contrast, a substantial reduction in the cofactor concentration (probably below 1 $\mu$M) would be required for the MTase to promote C to U conversions. Hence, the relative contributions of these two enzymatic processes to mutagenesis are likely to be determined by a variety of factors, including the levels of SAM and SAH, and the relative efficiencies of various DNA repair processes.

5MeC to T conversions mediated by C5 MTases could also contribute to mutations in nondividing cells. Most terminally differentiated cells in animal tissue do not divide but contain low levels of MTase activity. As there is little DNA synthesis in such cells, the MTase may have significant opportunity to revisit previously methylated sites to induce deamination. In this case, enzyme-mediated deamination is likely to supplement the spontaneous hydrolytic deamination of C and 5meC and help saturate the DNA repair processes. In certain tissues such as adult neurons, the activities of DNA-metabolizing enzymes, including UDG, are known to be very low (Focher et al., 1990). If this is true of other DNA mismatch correction processes as well, any uracil or thymine generated through deaminations is likely to persist.

*5MeC to T Conversion in Neurospora.* The proposal that C5 MTases may convert C to U or T was first proposed in the context of a phenomenon in *Neurospora* called repeat-induced point mutation (RIP) (Selker, 1990). RIP detects sequence duplications in the sexual phase of the *Neurospora crassa* life cycle and causes numerous C·G to T·A mutations and, typically, heavy localized methylation of cytosines in DNA (Selker et al., 1993). A similar process has been proposed to affect the distribution of CpG dinucleotides in mammalian genomes (Kricker et al., 1992). Selker suggested that C5 MTase that methylates the regions of sequence duplications could also cause C to U to T or C to 5meC to T conversions. At least four C5 MTases have been shown to be capable of C to U conversions (Bandaru et al., 1995; Shen et al., 1992; Wyszynski et al., 1994; Yang et al., 1995). A C to 5meC to T conversion in a single catalytic cycle of a C5 MTase has not been demonstrated, and some evidence has been presented against it (Smith et al., 1992). However, we have shown here that a C5 MTase is capable of converting 5meC in DNA to T, and hence, this reaction could underlie the rapid accumulation of mutations during RIP.

The experiments described above extend the range of chemical reactions found to be carried out by C5 MTases. They also suggest an alternate mechanism by which mutational hot spots at sites of cytosine methylation can arise. The precancer cell in which excess MTase does not cause excess methylation may be exactly the kind of physiological state in which MTase-mediated 5meC to T conversions may occur.

## ACKNOWLEDGMENT

S. Gabbara performed some of the early experiments in this study and made the initial observations regarding MTase-mediated 5meC to T conversion. It is a pleasure to acknowledge discussions regarding this project with him and with M. Wyszynski. We thank E. Selker (University of Oregon) and J. Christman (University of Nebraska School of Medicine) for valuable comments on the manuscript.

## SUPPORTING INFORMATION AVAILABLE

Reversion frequency and inhibition data upon which Figures 3 and 4 are based (2 pages). Ordering information is given on any current masthead page.

## REFERENCES

Bandaru, B., Wyszynski, M., & Bhagwat, A. S. (1995) *J. Bacteriol. 177*, 2950−2952.
Bestor, T. H., & Verdine, G. L. (1994) *Curr. Opin. Cell Biol. 6*, 380−389.
Brennan, S. O., Arai, K., Madison, J., Laurell, C.-B., Galliano, M., Watkins, S., Peach, R., Myles, T., George, P., & Putnam, F. W. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 3909−3913.
Brown, T. C., & Jiricny, J. (1987) *Cell 50*, 945−950.
Chen, L., MacMilllan, A. M., Chang, W., Ezaz-Nikpay, K., Lane, W. S., & Verdine, G. L. (1991) *Biochemistry 30*, 11018−11025.
Christman, J. K., Chen, M. L., Sheikhnejad, G., Dizik, M., Abileah, S., & Wainfan, E. (1993) *J. Nutr. Biochem. 4*, 672−680.
Cooper, D. N., & Youssoufian, H. (1988) *Hum. Genet. 78*, 151−155.
Coulondre, C., Miller, J. H., Farabaugh, P. J., & Gilbert, W. (1978) *Nature 274*, 775−780.
Dar, M. E., & Bhagwat, A. S. (1993) *Mol. Microbiol. 9*, 823−833.
Dizik, M., Christman, J. K., & Wainfan, E. (1991) *Carcinogenesis 12*, 1307−1312.
Doi, T., Kawata, T., Tadano, N., Iijima, T., & Maekawa, A. (1989) *J. Nutr. Sci. Vitaminol. 35*, 1−9.
El-Deiry, W. S., Nelkin, B. D., Celano, P., Yen, R.-W. C., Falco, J. P., Hamilton, S. R., & Baylin, S. B. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 3470−3474.
Eloranta, T. O. (1977) *Biochem. J. 166*, 521−529.
Feinberg, A. P., Gehrke, C., Kuo, K. C., & Ehrlich, M. (1988) *Cancer Res. 48*, 1159−1161.
Feo, F., Garcea, R., Daino, L., Pascale, R., Pirisi, L., Frassetto, S., & Ruggiu, M. E. (1985) *Carcinogenesis 6*, 1713−1720.
Focher, F., Mazzarello, P., Verri, A., Hubscher, U., & Spadari, S. (1990) *Mutat. Res. 237*, 65−73.
Friedman, S. (1985) *J. Biol. Chem. 260*, 5698−5705.
Friedman, S., & Ansari, N. (1992) *Nucleic Acids Res. 20*, 3214−3248.
Gabbara, S., Wyszynski, M., & Bhagwat, A. S. (1994) *Molec. Gen. Genet. 243*, 244−248.
Gabbara, S., Sheluho, D., & Bhagwat, A. S. (1995) *Biochemistry 34*, 8914−8923.
Garcea, R., Pascale, R., Daino, L., Frassetto, S., Cozzolino, P., Ruggiu, M. E., Vannini, M. G., Gaspa, L., & Feo, F. (1987) *Carcinogenesis 8*, 653−658.
Giannelli, F., Green, P. M., High, K. A., Lozier, J. N., Lillicrap, D. P., Ludwig, M., Olek, K., Reitsma, P. H., Goosens, M., Yoshioka, A., Sommer, S., & Brownlee, G. G. (1990) *Nucleic Acids Res. 18*, 4053−4059.
Goelz, S. E., Vogelstein, B., Hamilton, S. R., & Feinberg, A. P. (1985) *Science 228*, 187−190.
Greenblatt, M. S., Bennett, W. P., Hollstein, M., & Harris, C. C. (1994) *Cancer Res. 54*, 4855−4878.
Hayatsu, H. (1976) *Prog. Nucleic Acid Res. Mol. Biol. 16*, 75−124.
Henning, S. M., McKee, R. W., & Swedseid, M. E. (1989) *J. Nutr. 119*, 1478−1482.
Hibasami, H., Hoffman, J. L., & Pegg, A. E. (1980) *J. Biol. Chem. 255*, 6675−6678.
Hirschhorn, R., Tzall, S., & Ellenbogen, A. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 6171−6175.
Jones, P. A., Rideout, W. A., III, Shen, J.-C., Spruck, C. H., & Tsai, Y. C. (1992) *BioEssays 14*, 33−36.
Kautiainen, T. L., & Jones, P. A. (1986) *J. Biol. Chem. 261*, 1594−1998.
Koeberl, D. D., Bottema, C. D. K., Ketterling, R. P., Bridge, P. J., Lillicrap, D. P., & Sommer, S. S. (1990) *Am. J. Hum. Genet. 47*, 202−217.
Kricker, M. C., Drake, J. W., & Radman, M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 1075−1079.

Laird, P. W., & Jaenisch, R. (1994) *Hum. Mol. Genet. 3*, 1487−1495.
Laird, P. W., Jackson-Grusby, L., Fazelli, A., Dickinson, S., Jung, W. E., Li, E., Weinberg, R. A., & Jaenisch, R. (1995) *Cell 81*, 197−205.
Lieb, M. (1991) *Genetics 128*, 23−27.
Lindahl, T. (1993) *Nature 362*, 709−715.
Lindahl, T., & Nyberg, B. (1974) *Biochemistry 13*, 3405−3410.
Neddermann, P., & Jiricny, J. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 1642−1646.
Newberne, P. M., & Rogers, A. E. (1986) *Annu. Rev. Nutr. 6*, 407−432.
Pascale, R. M., Marras, V., Simile, M. M., Daino, L., Pinna, G., Bennati, S., Carta, M., Seddaiu, M. A., Massarelli, G., & Feo, F. (1992) *Cancer Res. 52*, 4979−4986.
Perutz, M. F. (1990) *J. Mol. Biol. 213*, 203−206.
Poirer, L. A., Wilson, M. J., & Shivapurkar, N. (1986) in *Biological Methylation and Drug Design* (Borchardt, R. T., Creveling, C. R., Ueland, P. M., Eds.) pp 151−161, Humana Press, Clifton, NJ.
Rouleau, J., MacLeod, A. R., & Szyf, M. (1995) *J. Biol. Chem. 270*, 1595−1601.
Santi, D. V., Garrett, C. E., & Barr, P. J. (1983) *Cell 33*, 9−10.
Selker, E. (1990) *Annu. Rev. Genet. 24*, 579−613.
Selker, E. U., Fritz, D. Y., & Singer, M. J. (1993) *Science 262*, 1724−1728.
Shapiro, R. (1981) in *Chromosome Damage and Repair* (Seeberg, E., Kleppe, K., Eds.) pp 3−18, Plenum Press, New York.
Shen, J.-C., Rideout, W. M., III, & Jones, P. (1992) *Cell 71*, 1−20.
Shen, J.-C., Rideout, W. M., III, & Jones, P. A. (1994) *Nucleic Acids Res. 22*, 972−976.
Shivapurkar, N., & Poirer, L. A. (1983) *Carcinogenesis 4*, 1051−1057.
Simile, M. M., Pascale, R., De Miglio, M. R., Nufris, A., Daino, L., Seddaiu, M. A., Gaspa, L., & Feo, F. (1994) *Cancer Lett. 79*, 9−16.
Simon, D., Grunert, F., von Acken, U., Doring, H. P., & Kroger, H. (1978) *Nucleic Acids Res. 5*, 2153−2167.
Smith, S. S. (1994) *Prog. Nucleic Acid Res. Mol. Biol. 49*, 65−111.
Smith, S. S., Kaplan, B. E., Sowers, L. C., & Newman, E. M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 4744−4748.
Sowers, L. C., Sedwick, W. D., & Shaw, B. R. (1989) *Mutation Res. 215*, 131−138.
Wainfan, E., & Poirier, L. A. (1992) *Cancer Res. 52* (Suppl. 7), 2071−2077S.
Wu, J. C., & Santi, D. V. (1987) *J. Biol. Chem. 262*, 4778−4786.
Wu, J., Issa, J.-P., Herman, J., Bassett, J. D. E., Nelkin, B. D., & Baylin, S. B. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8891−8895.
Wyszynski, M. W., Gabbara, S., Kubareva, E. A., Romanova, E. A., Oretskaya, T. S., Gromova, E. S., Shabarova, Z. A., & Bhagwat, A. S. (1993) *Nucleic Acids Res. 21*, 295−301.
Wyszynski, M., Gabbara, S., & Bhagwat, A. S. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 1574−1578.
Yang, A. S., Shen, J.-C., Zingg, J.-M., Mi, S., & Jones, P. A. (1995) *Nucleic Acids Res. 23*, 1380−1387.
Yokoyama, S., Sells, M. A., Reddy, T. V., & Lombardi, B. (1985) *Int. J. Cancer 45*, 2834−2842.

BI951687G