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DNA Methylase Induced by Bacteriophage φX174

(*E. coli* B infected by φX174/cytosine methylase/5-methylcytosine/DNA methylation)

AHARON RAZIN

Department of Cellular Biochemistry, The Hebrew University-Hadassah Medical School, Jerusalem, Israel, 91000

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ABSTRACT **A cytosine-specific DNA methylase activity, which is normally absent in the *Escherichia coli* B strain, was found to be induced in these cells by infection with bacteriophage φX174. *In vivo* experiments revealed a single 5-methylcytosine residue in the phage DNA molecule and 5-methylcytosine residues in the infected host DNA, in addition to the 6-methylaminopurine residues present in the uninfected cells. *In vitro*, a partially purified enzyme from infected cells methylated DNA from uninfected cells, but showed no activity with cellular DNA from infected cells. The partially purified methylase derived from uninfected cells lacks this activity.**

A single methylated cytosine is present in a nonrandom distribution in the single-stranded DNA molecule of bacteriophage φX174 (1). The methylation of the viral DNA takes place during the last stage of the DNA replication, with the replicative intermediates serving as substrate (2). These characteristics of the phage DNA methylation suggest an important biological role for the single methyl group present in this DNA (1, 2). The methyl group is probably not the product of a modification enzyme, since the host, *Escherichia coli* C, is devoid of the restriction modification system (3).

In order to elucidate the biological significance of the methylation of phage DNA, it was of special importance to find out whether the host or the virus specifies the enzyme responsible for this methylation. The DNA of the host, *E. coli* C, contains both 5-methylcytosine (m^5Cyt) and 6-methylaminopurine (m^6Ade) (4, 5). The possibility, therefore, exists that the host methylase methylates phage DNA.

E. coli B cells are known to be devoid of cytosine methylase activity, and their DNA contains only m^6Ade as a minor base (4, 5, 11, 12, 16, 17). This feature makes these cells an ideal tool to study the genetic information directing cytosine-specific methylation in genomes that can be introduced into the cells. Mamelak and Boyer (11) introduced the gene controlling the methylation of cytosine in *E. coli* K12 into *E. coli* B cells. The hybrid recombinant was found to methylate DNA similarly to *E. coli* K12 with respect to cytosine methylation. Hattman *et al.* (16) observed high contents of m^5Cyt in *E. coli* B DNA after transfer of the (N3) drug-resistance factor. The appearance of cytosine methylase activity in *E. coli* B harboring the (N3) factor was confirmed by *in vitro* experiments (17).

E. coli B could, therefore, provide a good system for investigating the existence of a methylase induced by φX174 phage. This kind of study became possible with the isolation, by Dr. David Denhardt, of a new mutant of φX174 capable of infecting *E. coli* B. The present report describes experiments which show that infection of *E. coli* B with Denhardt's mutant of the φX174 virus gives rise to the appearance of a cytosine-specific DNA methylase in the host.

Abbreviations: m^5Cyt, 5-methylcytosine; m^6Ade, 6-methylaminopurine.

MATERIALS AND METHODS

Bacteria and Bacteriophage. E. coli B served as the host in all the experiments discussed in this report. A mutant of φX174 (φxe$^-$h8h10), capable of infecting *E. coli* B, was isolated by Dr. Denhardt, and kindly provided for this study: the designation φX174/B will be used here.

Media. The growth media TPA and TPG (7) used in the present study were supplemented with 25 μg/ml of adenosine and 10 μg/ml of thymine.

Large-scale Preparation of the Mutant φX174/B. The yield of the mutant phage was found to be low when *E. coli* B cells were grown and infected in TPA medium; the yield was negligible in TPG medium. Certain L-amino acids were found to facilitate phage production. The highest phage yield was observed with 50 μg/ml of L-leucine added to TPG medium. Addition of L-methionine or a combination of L-methionine and L-cytosine at the above concentration had an inhibitory effect on the phage production.

Isolation and Digestion of Phage [methyl-^{3}H]DNA. The labeling *in vivo* of phage DNA, its isolation from progeny phage particles, the digestion of the isolated [*methyl-^{3}H*]DNA, and chromatography of the bases were done as described (1) with the following modifications: *E. coli* B cells were grown in TPG medium supplemented with 25 μg/ml of adenosine, 10 μg/ml of thymidine, and 50 μg/ml of L-leucine. At a cell concentration of 5×10^8 cells per ml, the culture was infected with φX174/B (multiplicity of infection = 2). 5 min after infection, 5 mCi/liter of [*methyl-^{3}H*]methionine (6.4 Ci/mmol) (Amersham, England) was added to the infected culture. 2 hr after infection the cells were centrifuged, washed, and suspended in 0.05 M sodium tetraborate. The cell suspension contained 90% of the phage yield. The cells were lysed; the virus was banded in CsCl and further purified on a neutral sucrose gradient. The DNA was extracted from the phage particles with phenol and purified by subsequent alcohol precipitations. The purified DNA was digested with trifluoroacetic acid, and the bases were chromatographed and analyzed (1).

TABLE 1. *Methylation pattern of the φX174/B DNA in vivo*

Experiment no.	Methyl density*	
	m^5Cyt	m^6Ade
I	1.23	0.69
II	1.1	0.8
III	1.2	0.4

* Methyl density is defined by the number of methyl groups per one molecule of phage DNA. It was calculated as described (Exp. type 1 in ref. 1).

Isolation of Host [methyl-^3H]DNA. Host DNA of infected and uninfected cells was purified from the pellet obtained through the isopycnic centrifugation of the cell lysate. The DNA was digested with TFA medium and analyzed as before (1).

Preparation of Partially Purified Methylase. Methylases from infected and uninfected cells were partially purified by the procedure described by Kay *et al.* (8). Frozen cells were ground to a sticky paste with twice their weight of alumina 305. The mixture was further ground with a volume equal to the original weight of the cells in 20 mM Tris·HCl buffer (pH 7.4) containing 20 mM $MgCl_2$. Solid $(NH_4)_2SO_4$ (164 mg/ml) was added to the 35,000 × *g* supernatant to achieve 30% saturation. The supernatant was collected as above and 181 mg/ml of $(NH_4)_2SO_4$ was added to obtain a 60% saturation. The resulting precipitate was dissolved in 0.01 M Tris·HCl, (pH 7.4) and kept at −20°. The 30–60% ammonium sulfate fraction showed low background with no DNA.

Assay of Methylase Activity. The reaction mixture contained, in a final volume of 125 μl, the following ingredients: 12.5 μmol of Tris·HCl buffer (pH 8), 0.5 μmol of $MgCl_2$, 0.5 μmol of dithiothreitol, 10 nmol of [*methyl*-^{3}H]*S*-adenosyl-methionine (50 Ci/mol) (Amersham, England), 25 μg of DNA substrate, and 500–750 μg of protein of the 30–60% ammonium sulfate methylase preparation. The reaction mixture was incubated for 30 min at 37°. The reaction was terminated by the addition of 0.5 ml of 0.5 N NaOH–1% sodium dodecyl sulfate and heating for 15 min at 80°. The DNA was precipitated by adding 5 ml of 10% trichloroacetic acid, washed on GF/C filters, and counted in toluene–PPO–POPOP scintillation fluid in a Packard liquid scintillation counter. Results were corrected for the background without DNA.

RESULTS

In Vivo Methylation of φX174/B DNA. The methylation pattern of φX174 bacteriophage DNA in *E. coli* B is shown in Table 1. One m^5Cyt is found per phage DNA molecule. In this respect, the methylation of phage DNA in *E. coli* B is identical to that found in *E. coli* C (1). The label in m^6Ade varied in different experiments and represented in all instances less than one methyl group per molecule of phage DNA. It is therefore unlikely that the observed m^6Ade is a product of the modification methylase present in *E. coli* B (6).

TABLE 2. *Methylation pattern of host DNA in vivo*

Experiment no.	Uninfected *E. coli* B		Infected *E. coli* B	
	m^5Cyt	m^6Ade	m^5Cyt	m^6Ade
	cpm/50 μg of DNA			
I	0	1660	238	1810
II	0	2020	170	2000

TABLE 3. *In vivo methylation by methylase from infected and uninfected E. coli B cells*

Source of methylase	Source of DNA		
	Uninfected *E. coli* B	Infected *E. coli* B	Calf thymus
	pmol incorporated/hr per mg of protein		
Uninfected *E. coli* B	0.2	0.3	62.6
Infected *E. coli* B	10.4	0.0	68.4

In Vivo Methylation of Host DNA. The pattern of methylation of infected and uninfected *E. coli* B DNA is described in Table 2. While DNA isolated from uninfected *E. coli* B cells contains m^6 Ade but not m^5Cyt, in the DNA isolated from infected cells both adenine and cytosine residues were methylated. The extent of methylation of adenine residues is not altered by the infection, whereas in response to infection m^5Cyt appears in addition to the m^6Ade normally present in the DNA. The ratio m^6Ade/m^5Cyt is about 10:1, whereas an m^6Ade/m^5Cyt ratio of 1.5:1 is found in *E. coli* C DNA (12).

In Vitro Methylation of Host DNA. DNA methylase was partially purified from cell extracts of infected and uninfected *E. coli* B. Its activity was tested with cellular DNA, derived from either uninfected or infected *E. coli* B cells as substrate. Table 3 summarizes the results of these assays. Methylase isolated from uninfected cells, as expected, did not methylate host DNA. On the other hand, the partially purified enzyme from infected cells methylated uninfected *E. coli* B DNA, but no methylation took place when infected host DNA was used as the substrate.

DISCUSSION

The present study demonstrates that *E. coli* B cells acquire cytosine-specific DNA methylase activity upon infection with the bacteriophage φX174. This conclusion is drawn from the methylation patterns of phage DNA (Table 1) and host DNA (Table 2) and the capacity of extracts of infected *E. coli* B cells to methylate DNA *in vitro* derived from uninfected cells (Table 3). These results suggest that the bacteriophage controls a cytosine-specific DNA methylase. These data seem to lend further support to the previously drawn conclusion (2) that the methylation process plays an essential role in the maturation of the virus.

The molecular basis of the phage-induced activity remains to be elucidated. The available data do not permit a decision as to whether the phenomenon concerned reflects a capacity of the viral genome to code for the specific enzyme protein or rather some indirect mechanism, such as derepression of the host genome or an alteration of the substrate specificity of the preexisting host enzyme.

It may be pertinent to mention in this connection that the modification methylase isolated from *E. coli* B by Lautenberger and Linn (9) has been shown to consist of two non-

identical polypeptides with an overall molecular weight of about 115,000 d. Cistrons coding for a molecule of this size, by the ϕX174 phage, would require an incredibly large portion of the viral genome and, therefore, appear unlikely. It is, however, still possible that the virus determines a polypeptide subunit that plays a role in the recognition of the site of methylation on the phage DNA, and this subunit acts in concert with the host methylase.

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