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Analysis of Bacteriophage Deoxyribonucleic Acid Sequences Methylated by Host- and R-Factor-Controlled Enzymes

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Phages λ and fd were propagated in *Escherichia coli* strains that have either host K-12 or the N-3 R-factor deoxyribonucleic acid-cytosine methylase activity. Pyrimidine tracts containing ^{3}H-labeled 5-methylcytosine (MeC) were analyzed; in all cases, the major methylated sequence was 5′ . . . C-MeC-T . . . 3′.

Deoxyribonucleic acid (DNA)-cytosine methylases are controlled by both *Escherichia coli* K-12 bacteria (5) and by certain drug resistance R-factors (3, 6; R. Yoshimori, Ph.D. thesis, University of California, San Francisco, 1971). DNA-cytosine methylation is the basis for the host-specificity system (*hsp*II or RII; 1, 2, 13, 14; Yoshimori, Ph.D. thesis, 1971) controlled by the R-factors, N-3 and R15 (3, 10), whereas the bacterial enzyme has no known biological function. We have proposed that the host and R-factor enzymes methylate very similar nucleotide sequences in phage DNA (9). In those experiments, we propagated phages fd and λ in various strains of *E. coli* in the presence of [*methyl*-^{3}H]methionine. The purified phage DNA was depurinated (4, 11), and the resulting oligopyrimidine tracts were fractionated according to chain length and base composition. We found that in fd DNA isolated from phage propagated in either *E. coli* K-12 *mec*$^+$ cells (which have host cytosine methylase activity) or in *mec*$^-$ (N-3) cells (which lack host cytosine methylase [7] but have N-3 plasmid methylase activity), [5-^{3}H]methylcytosine (MeC) was present only in the (C_2T) tripyrimidine tract; the parenthesis indicates that the tract contains a mixture of sequence isomers. For λ propagated in *mec*$^+$ or in *mec*$^-$ (N-3) cells, the major [^{3}H]MeC-containing oligopyrimidine tract was (C_2T).

In the present communication we report the base sequence of the MeC-containing tripyrimidine. Labeled tracts from λ or fd phage DNA were prepared as above and analyzed according to the general scheme outlined in Fig. 1. First, the [^{3}H]MeC-containing tract, p(CpCpT)p, was treated with bacterial alkaline phosphatase to remove the terminal phosphate groups. The labeled trinucleoside-diphosphate digestion product was isolated by paper electrophoresis and subjected to three different enzyme digestions: (A) total spleen phosphodiesterase digestion to determine if any [^{3}H]MeC is located in the 3′-terminal residue (released as a nucleoside); (B) total snake venom phosphodiesterase digestion to determine if any [^{3}H]MeC is located in the 5′-terminal residue (released as a nucleoside); and (C) partial snake venom phosphodiesterase digestion to determine which 5′-terminal dinucleoside-monophosphate(s) contains [^{3}H]MeC. The results of these experiments are summarized in Table 1.

After the total spleen and venom phosphodiesterase digestions (Table 1, columns A and B), almost all the [^{3}H]MeC label was found in the mononucleotide. We conclude that only a minor fraction of the [^{3}H]MeC is in the 3′- or 5′-terminal residue; therefore, the [^{3}H]MeC must be located in the middle residue, within the sequence(s) C-MeC-T and/or T-MeC-C. The results of the partial snake venom digestions (Table 1, column C) reveal that most of the [^{3}H]MeC is contained in the CpC-dinucleoside-monophosphate. Therefore, C-MeC-T is the major methylated sequence; this is true for both λ and fd DNA isolated from phage grown in *mec*$^+$ or *mec*$^-$ (N-3) cells.

These results are in harmony with those of Boyer et al. (3), who determined the sequence specificity of the RII DNA-cytosine methylase controlled by the R15 plasmid. The double-stranded sequence methylated in vitro by the purified methylase was proposed to be

$$5' \ldots N_1 C C^* T G G \ldots 3'$$

$$3' \ldots GG AC^* C N_2 \ldots 5'$$

where the N_1 and N_2 bases are not specific and the asterisk represents the MeC residue. For phage λ DNA methylated in vivo, we proposed that N_1 is usually a purine (Pu) (9). The results presented in this communication suggest that the pentanucleotide PuCC*T Pu is the major

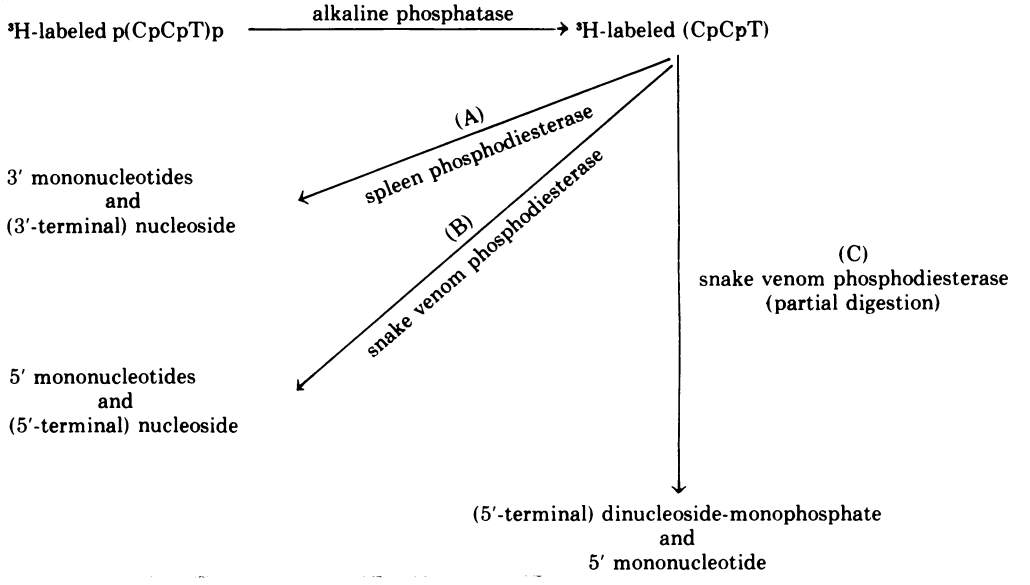

FIG. 1. *Analysis of the location of [³H]MeC in the (C_2T) tripyrimidine tract. The [³H]MeC-labeled p(CpCpT)p tract (~0.3 mg; 2,000 counts/min) was digested with ~0.03 mg of heated (8) E. coli alkaline phosphatase [in 0.5 ml of 0.02 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8 at 37 C for 1 h]. The digest was evaporated to dryness, suspended in water and spotted onto Whatman 3 MM paper (2.5 by 57 cm) together with ~0.05 mg each of CpCpC and p(CpCpT)p markers. After paper electrophoresis in a water-cooled flate-plate apparatus (in 0.1 M Tris-hydrochloride, pH 8) at 2 kV for 1.25 h, the ³H-labeled tri-nucleoside-diphosphate was eluted, evaporated to dryness, and subjected to the following. (A) Total spleen phosphodiesterase digestion: the ³H-labeled (CpCpT) (~200 counts/min) and 0.15 mg of carrier CpCpC were digested with 2.0 units of spleen phosphodiesterase (in 0.5 ml of 0.05 M sodium acetate, pH 5.5, 0.001 M disodium ethylenediaminetetraacetic acid, 0.05% [vol/vol] Tween 80). After 2 h at 37 C the mixture was evaporated to dryness and subjected to paper electrophoresis as described above. The nucleoside and nucleotide spots were extracted and counted for ³H radioactivity. (B) Total snake venom phosphodiesterase digestion: ³H-labeled (CpCpT) (~200 counts/min) and 0.15 mg of carrier CpCpC were digested with ~25 μg of purified (12) snake venom phosphodiesterase (in 0.5 ml of 0.05 M Tris-hydrochloride [pH 8.9], 0.01 M $MgCl_2$ at 37 C for 2 h). The nucleoside and nucleotides were separated and analyzed as in (A). (C) Partial snake venom phosphodiesterase digestion: ³H-labeled (CpCpT) (~600 counts/min) and 0.15 mg of carrier CpCpC were digested with 2.5 μg of purified snake venom phosphodiesterase (in 0.5 ml of 0.05 M Tris-hydrochloride, pH 8.9, and 0.01 M $MgCl_2$ at 37 C for 0.5 h). The digestion products (dinucleoside monophosphate, nucleotide, nucleoside, and undigested tract) were separated in two successive paper electrophoresis runs: (i) in 0.1 M Tris-hydrochloride, pH 8.0 (at 2 kV for 1.25 h), to isolate the [³H]dinucleoside monophosphates (CpC and (TpC)), and then (ii) in 0.1 M ammonium formate, pH 3.2 (at 2.5 kV for 1.25 h), to separate the dinucleoside-monophosphates according to base composition. In this electrophoresis run, markers UpC (which should co-electrophorese with CpT and TpC) and CpC were included. The relevant regions were eluted and counted for ³H radioactivity. (The enzymes used above were from Worthington Biochemicals; the marker oligonucleotides were from P-L Biochemicals.)*

TABLE 1. *Location of [^{3}H]MeC in tripyrimidine (CpCpT) tracts*[a]

DNA	Fraction of [^{3}H]MeC label in[b]		
	(A) 3′ Nucleotides after spleen phosphodiesterase	(B) 5′ Nucleotides after venom phosphodiesterase	(C) 5′-Terminal CpC after partial venom phosphodiesterase[c]
fd·*mec*$^-$ (N-3)	0.9	0.9	1.0
fd·*mec*$^+$	0.8	0.9	0.7
λ·*mec*$^-$ (N-3)	0.8	0.9	0.8
λ·*mec*$^+$	0.8	0.9	0.7

[a] See legend to Fig. 1 for details of methods.

[b] The values presented are an average of 2 to 3 independent determinations; the variation was ±10% of the mean.

[c] Fraction in CpC/total dinucleoside monophosphate.

sequence methylated in λ DNA by both the N-3 and *mec*$^+$ DNA-cytosine methylases. For phage fd propagated in *mec*$^+$ and *mec*$^-$ (N-3) cells, the only MeC-containing sequence in mature, single-stranded viral DNA is . . . Pu C C* T Pu . . . (this presumably corresponds to the N_1-containing strand, where N_1 = Pu).

The results reported here lend further credence to our hypothesis that the host *E. coli* K-12 *mec*$^+$ and RII DNA-cytosine methylases possess similar sequence specificity (9). In this regard, we have recently shown that a variety of phage and cell DNAs methylated in vivo by the *mec*$^+$ enzyme are protected against in vitro cleavage by purified RII-restriction nuclease (S. G. Hughes and S. Hattman, submitted for publication; S. Schlagman, S. Hattman, M. S. May, and L. Berger, in preparation). Thus, the *mec*$^+$ methylase behaves as an RII modification enzyme, and it is likely that the *mec*$^+$ and the RII enzymes methylate the same sites on phage and bacterial DNAs.

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