JOURNAL OF BACTERIOLOGY, Apr., 1966
Vol. 91, No. 4
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Methylated Bases in the Host-Modified Deoxyribonucleic Acid of *Escherichia coli* and Bacteriophage λ[1]

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Received for publication 27 September 1965

ABSTRACT

GOUGH, MICHAEL (Brown University, Providence, R.I.), AND SEYMOUR LEDERBERG. Methylated bases in the host-modified deoxyribonucleic acid of *Escherichia coli* and bacteriophage λ. J. Bacteriol. **91**:1460–1468. 1966.—The deoxyribonucleic acid (DNA) from strains of *Escherichia coli* and phage λ was examined to determine whether the types or amounts of methionine-derived methylated bases present correlated with the host-specific modification of that DNA. The DNA of strain C600 (which has K-12 modification specificity) and of a modificationless mutant of C600 are similar in their content of 5-methylcytosine and 6-methylaminopurine. Strains Bc251 and its P1-lysogen differ in P1-controlled specificity, but they have the same content of 6-methylaminopurine, and both lack 5-methylcytosine in their DNA. Phage λ contains the same methylated bases as its host of origin, but in reduced amounts and in different proportions. Although minor amounts of these methylated bases may have importance as a result of their location, the presence of the majority of these methylated bases is irrelevant to the specificity of host modification of DNA.

A host-controlled or host-induced modification is a change in bacteriophage host range which depends on the last bacterial host for the phage (32). Bacteriophage with a given host-controlled specificity may be rejected or accepted upon infection of a new host. When a bacteriophage succeeds in multiplying in a new host, the host specificity of that strain may be acquired by the newly made phage. Different types of host specificity are found in *Escherichia coli* strains B, C, and K-12 (4, 6). A fourth type of specificity which may be acquired independently and additively by these strains is controlled by the prophage P1 (3, 27). In Fig. 1, the host specificity of these strains is described in terms of the efficiency of infection of these bacteria by phage λ. The host-specific patterns of restriction and modification which apply to certain phages can also operate to reduce the fertility of matings between these bacterial strains. Thus, a strain which restricts an infection by bacteriophage grown on another host is relatively infertile as a recipient for the transfer of a bacterial chromosome from that host (3, 5, 7, 8, 28, 37).

Restriction and modification of phage involve two host activities: one imparts an identification of the host of origin to phage; the other screens for this host of origin. These activities are exerted at the level of the phage deoxyribonucleic acid (DNA) molecule, since phenol-extracted DNA of phage λ (24) exhibits the same host specificity as the free phage in regard to infectivity (17). Moreover, this host specificity can be imparted to an already-formed DNA molecule, since, under certain conditions of infection, conserved phage DNA can acquire a new modification specificity (3).

Enzymatic mechanisms have been proposed for host-controlled restriction and modification (27, 28). In these models, modification of DNA is visualized as a protection against host-specific screening activity. Protective modification carried by DNA might operate directly by conferring resistance on specific DNA sites otherwise susceptible to host-specific nucleases. Alternatively, it

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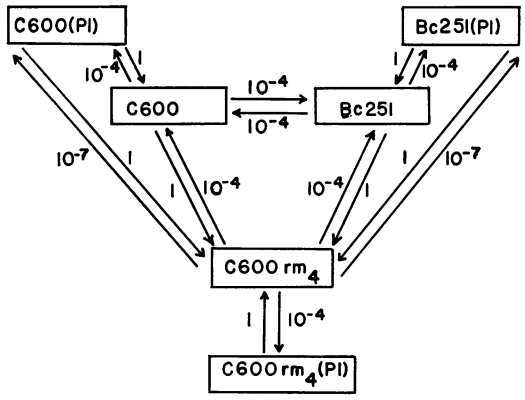

FIG. 1. *Host-controlled restriction and modification in strains of Escherichia coli. The arrows represent infections of one strain by phage λ grown on another. The associated numbers indicate the approximate relative efficiency of plating of phage λ for some of the different combinations of host-of-origin and plating host [adapted from Bertani and Weigle (6), Arber and Dussoix (2), Lederberg (28)]. Strains C600 and C600 rm4 have the properties of host restriction and modification characteristic of strains K-12 and C, respectively. Strain Bc251, with the host specificity of strain B, and Bc251 (P1) are used in the present work for study of P1-controlled specificity. A qualitatively similar scheme applies to the transfer of bacterial DNA in matings between these strains.*

might act indirectly in conjunction with host-specific agents to form complexes which are resistant to degrading activities present in all these strains.

The survival of host-controlled modification through the preparation of transforming DNA argues for consideration of the loss or gain of covalently bound groups as the molecular basis for modification of DNA. The methyl group is a suitable candidate for this function because of its known presence in minor quantities in some microbial DNA preparations (16), and because of the nuclease resistance conferred on some polynucleotides by methylation (18, 41). In addition, host specificities controlled by strain K-12 or prophage P1 were not imparted to phage λ propagated under methionine-deficient conditions (2; Lederberg, *unpublished data*).

The present report deals with an examination of the methylated bases and nucleosides in the DNA of phage λ and its host strains to learn whether a correlation existed between the pattern of methylation of DNA and host-controlled modification. A likely source of any methyl group which might be involved was thought to be *S*-adenosyl-L-methionine for which L-methionine is a precursor (11). This view was reinforced by the report on *S*-adenosyl-L-methionine dependent DNA methyl transferases (21). The systems chosen for this study were C600 and a mutant, C600 *rm*$_4$, possessing K-12 and C host specificity, respectively. Strains Bc251 and Bc251 (P1) were used as representatives of cells with and without P1-controlled specificity.

MATERIALS AND METHODS

Bacteria and bacteriophage. The following strains of *E. coli* were employed. [Abbreviations and symbols used for designations of genotype are: *thr*, threonine; *leu*, leucine; B_1, vitamin B_1; *met*, methionine; *ade*, adenine; *lac*, lactose; *mal*, maltose; +, capable of producing or utilizing; −, incapable of producing or using; *T6*^{r}, resistant to phage T6; *str*^{r}, resistant to streptomycin; (P1), lysogenic for phage P1; *rm*, restriction-modification mutant; *ind*$^-$, mutant of λ not inducible by ultraviolet light; c_{857}, mutant of λ forming clear plaques at 37 C, and inducible by heat shock at higher temperatures.] C600, *F*$^-$, *thr*$^-$, *leu*$^-$, B_1^-, *lac*$^-$ (1) has the host specificity characteristic of K-12 strains in that it restricts phage λ grown on strains with the host specificity of *E. coli* C (for example, C or C600 *rm*$_4$) or with the host specificity of *E. coli* B (for example, B or Bc251). This strain imparts to phage λ the ability to grow on strains having K-12 host specificity, such as K-12 or C600 (3, 6). C600 *rm*$_4$, a mutant of C600 lacking the restriction and modification activities of the parent strain [described as C600.4 by Meselson (36)], does not restrict phage λ, but λ grown on this mutant is restricted by strains of either K-12 host specificity or B host specificity. In this respect, strain C600 *rm*$_4$ has a host specificity identical to that of *E. coli* C. Bc251, a *F*$^-$, *mal*$^+$, λ-sensitive prototroph (4) derived from strain Bc (11*a*), a prophage-cured variant of strain B, has B host specificity in that it restricts phage λ grown on strains with the host specificity of *E. coli* C or K-12, and phage λ grown on this strain is restricted by K-12 strains.

T6-resistant derivatives of C600, C600 *rm*$_4$, and Bc251 were isolated as spontaneous mutants in order to use these strains for mating experiments reported elsewhere. Methionine-requiring auxotrophs, in turn, were derived from T6-resistant cultures treated with the mutagen, 1-methyl-3-nitro-1-nitrosoguanidine (33). Adenine-requiring auxotrophs of C600 *T6*^{r} *met*$^-$ and C600 *rm*$_4$ *T6*^{r} *met*$^-$ were obtained from cultures irradiated with ultraviolet light. The P1kc variant of phage P1 (30), which imposes an additional restriction and modification on phage (3, 27), was used to derive P1-lysogens from Bc251 *T6*^{r} and Bc251 *T6*^{r} *met*$^-$.

For the preparation of labeled phage λ, lysogens were derived by use of the heat-inducible mutant, λ *ind*$^-$ c_{857} (40). These lysogens are induced to lyse and liberate phage by a short exposure of the cells to 44 C. The host specificity characteristics of this mutant λ are the same as those of wild type λ.

Media. Broth was 1% tryptone (Difco) with 0.5% NaCl. Tryptone agar and soft tryptone agar contained broth with 1% agar (Difco or Oxoid No. 3) and 0.6%

agar (Difco), respectively. PN salts contained 0.3% K_2HPO_4, 0.04% KH_2PO_4, 0.2% $(NH_4)_2SO_4$, and 0.012% $MgSO_4$. PNG medium was PN salts with 0.04% glucose. PNG agar contained PNG with 1% agar (Difco Noble). When necessary for growth, PNG medium or PNG agar was supplemented with 20 mg of L-threonine, 20 mg of L-leucine, 1 to 2 mg of L-methionine, 100 mg of adenine, and 1 mg of thiamine hydrochloride per liter. For adenine prototrophs, 100 mg of adenine, or 50 mg of adenine and 50 mg of guanosine were added per liter of PNG medium to prevent the synthesis of any labeled adenine and guanine, detectable amounts of which are otherwise formed.

Growth responses of the methionine auxotrophs. A 1-mg amount of L-methionine per liter of PNG medium permitted the growth of 2.2×10^{11} cells of all methionine-requiring auxotrophs, with doubling times of 51 to 62 min. For strains C600 *T6*^{r} *met*$^-$ and Bc251 *T6*^{r} *met*$^-$, DL-homocysteine (the purity of which was confirmed chromatographically in solvent ii described below) at 2 mg per liter of PNG medium permitted the growth of 10^{11} to 1.5×10^{11} cells with doubling times of 3 to 4 hr. At concentrations of 20 mg of DL-homocysteine per liter, the doubling times approximated that with 1 mg of L-methionine. No growth response was seen with 10 μg of vitamin B_{12} per liter. These two mutants appear to be blocked before the synthesis of homocysteine. The methionine requirement of C600 *rm*$_4$ *T6*^{r} *met*$^-$ was satisfied by 10 to 100 μg of vitamin B_{12} per liter, but not by 20 mg of DL-homocysteine. This mutant is probably blocked in the synthesis of vitamin B_{12}.

Preparation of bacterial DNA. A 40-ml amount of a culture of cells growing exponentially in PNG medium at 10^8 to 4×10^8 bacteria per milliliter was centrifuged. The cells were washed once in PN salts and resuspended in 100 ml of PN medium containing H^3- or C^{14}-methyl-labeled methionine (New England Nuclear Corp., Boston, Mass.). The bacteria were chilled and harvested after 1 to 3 generations, and the DNA was extracted and purified by the phenol method of Saito and Miura (38). The amount of DNA in the culture made during growth in the labeled medium, and in the final preparation, was determined by the diphenylamine (DPA) reaction of Burton (10).

Preparation of phage DNA. Bacteria lysogenic for λ *ind*$^-$ c_{857} were grown in 50-ml cultures at 30 C to a concentration of 2×10^8 to 4×10^8 bacteria per milliliter. The cells were warmed to 44 C for 11 min, then chilled, centrifuged, washed once, and resuspended at 37 C in 100 ml of PNG medium containing the desired label. The culture was incubated at 37 C for about 75 min, by which time lysis was complete. Bacterial debris was removed by centrifugation at $15,000 \times g$ for 10 min. Phage λ was collected and washed by two cycles of centrifugation at $70,000 \times g$ for 75 min, then purified by equilibrium centrifugation in isopycnic CsCl solution (43). DNA was liberated by shaking purified phage with redistilled phenol saturated with water at *p*H 8.5. The phenol was extracted from the aqueous phage with redistilled ether. Phage DNA was assayed by the DPA reaction.

Hydrolysis of DNA. Crude snake venom (*Crotolus adamanteus*, Ross Allen Snake Farms, Silver Springs, Fla.) was fractionated through the first acetone precipitation step of Williams, Sung, and Laskowski (44), and was purified further by the method of Keller (25). A 10-μg amount of this preparation [in 0.5- to 1-ml reaction volume containing 0.001 M $CaCl_2$, 0.01 M NaCl, and 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 9.0) plus a drop of chloroform] rendered 8 to 25 μg of DNA ethyl alcohol-soluble in 6 to 16 hr at 30 C. Three to eight units of purified bacterial alkaline phosphatase [a gift from Atsuo Nakata; a unit is that amount of enzyme which will produce a change in optical density at 410 mμ of 1 per minute at 37 C when the substrate is nitrophenolphosphate at 0.2 mg/ml in 1 M Tris buffer, *p*H 8.0 (20)] were then added, and the incubation was continued for 6 to 8 hr. Protein was precipitated with cold 5% perchloric acid (PCA) and discarded. The preparation was then neutralized, and reduced in volume to less than 0.01 ml by freeze-drying. The deoxyribosides in the final preparation were assayed with DPA and separated by chromatography.

Chemical hydrolysis was carried out by the method of Marshak and Vogel (35) with the use of 1 μliter of 70% PCA per 70 μg of DNA. When necessary, unlabeled carrier DNA was added to bring the total DNA to be hydrolyzed to 70 μg or more. After hydrolysis, the residue was extracted three times with 20 μliters of water, and the combined extracts were applied to Whatman 3MM chromatography paper.

Chromatography. The solvents used were (i) *n*-butanol-water (86:14, v/v) in troughs with 5% ammonium hydroxide in *n*-butanol-water in the bottom of the tank (34); (ii) 2-propanol-concentrated HCl-water [176:44:30, v/v/v (45)]; (iii) 2-propanol-concentrated HCl (176:44, v/v). Chromatographic separations were made with Whatman 3MM paper at 20 to 22 C. Ascending solvent fronts were allowed to migrate 15 to 20 cm; descending fronts moved 35 to 40 cm. Reference standards of adenine, cytosine, guanine, thymine, 5-methylcytosine (5MC), and 6-methylaminopurine (6MAP), or their deoxyribosides (Calbiochem) were included in each separation.

Measurement of radioactivity. Chromatograms were cut into strips, 2 cm wide and 1 cm in the direction of solute migration, and immersed in vials of scintillation solution [4 g of 2,5-diphenyloxazole and 0.05 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene]. Radioactivity was measured with a Packard Tricarb or Nuclear Chicago scintillation counter. Standards were prepared with measured amounts of H^3-methyl-methionine and C^{14}-methyl-methionine on Whatman 3MM chromatography paper.

Calculation of the content of methylated bases. For methionine prototrophs, it was assumed that the methyl transferases acting on cytosine and adenine residues used the same methyl donor pool. Therefore, the relative amounts of different methylated bases were computed from the relative amounts of radioactivity in the different regions of the chromatograms.

For methionine-requiring auxotrophs, it was fur-

TABLE 1. *Methylated bases in perchloric acid digests of the DNA of Escherichia coli**

Strain	Radioactivity (count/min) in chromatogram regions		Amt (moles × 10^{-9}) of labeled DNA hydrolyzed	Radioactivity (count per min per nmole of bases) in bases per unit of DNA		Ratio of 5MC-6MAP radioactivity
	5MC	6MAP		5MC	6MAP	
C600 *rm*$_4$ *T6*^{r}	348	596	159	2.2	3.7	0.58
C600 *T6*^{r}	151	260	60	2.5	4.3	0.58
Bc251 *T6*^{r}	0.3	523	90	0.003	5.8	<0.001
Bc251 *T6*^{r} (P1)	1.1	1380	288	0.004	4.8	<0.001

* Cells of all strains were grown under identical conditions in the presence of 1 μg per ml of C^{14}-methyl-labeled L-methionine (6.3 counts per min per picomole). The DNA was extracted and hydrolyzed with PCA as described in Materials and Methods. The liberated bases were separated by descending chromatography in solvent i followed by ascending chromatography in a second dimension in solvent ii. The amount of DNA made during the period of isotope labeling was determined by a diphenylamine assay.

ther assumed that the original specific activity of the input methionine was maintained during the incubation of cells with this supplement. This specific activity, the radioactivity of the chromatograms, and the amount of DNA synthesized in the presence of label permitted an estimation of the number of bases in DNA with methyl groups derived from methionine (see Discussion).

RESULTS

Methylated bases present in the DNA of E. coli. Methionine prototrophs *E. coli* C600 *T6*^{r}, C600 *rm*$_4$ *T6*^{r}, Bc251 *T6*^{r}, and Bc251 *T6*^{r} (P1) were grown in the presence of H^3- or C^{14}-methyl-labeled methionine. The extracted DNA was hydrolyzed with PCA; the products were separated by chromatography in solvent i followed by solvent ii. The results listed in Table 1 show that strains C600 *T6*^{r} and C600 *rm*$_4$ *T6*^{r} produced labeled 5MC and 6MAP in their DNA, and that the ratio of 5MC to 6MAP was about 0.6 in both strains. Strains Bc251 *T6*^{r} and Bc251 *T6*^{r} (P1) produced labeled 6MAP, but their labeled 5MC was less than 0.001 that of their 6MAP. No other labeled methylated base was detected in the DNA of these strains. Thus, although C600 *T6*^{r} and C600 *rm*$_4$ *T6*^{r} differ in having a host specificity of K-12 and C, respectively, the types of methylated bases and their amounts relative to each other are the same. Likewise, no difference is found in the types of methylated bases in the DNA of strains possessing and lacking P1-controlled specificity, i.e., Bc 251 *T6*^{r} (P1) and Bc251 *T6*^{r}. A qualitative difference in labeled methylated bases is found only when C600 *T6*^{r} and C600 *rm*$_4$ *T6*^{r} are compared with Bc251 *T6*^{r} which lacks 5MC in its DNA. The absence of 5MC in the DNA of derivatives of Bc251 is consistent with a report on its comparatively low level in strain B (12).

To determine the approximate levels of methylated bases in the DNA of these strains, the analyses performed with prototrophs were carried out on methionine-requiring auxotrophs. The results in Table 2 indicate that the amount of 6MAP is about 0.2% of all bases present in DNA for the strains listed here. In strains C600 *T6*^{r} *met*$^-$ *ade*$^-$ and C600 *rm*$_4$ *T6*^{r} *met*$^-$ *ade*$^-$, 5MC is about 0.12% of the DNA bases. The 5MC-6MAP ratio is about 0.6 for both of these auxotrophs. In strains Bc251 *T6*^{r} *met*$^-$ and Bc251 *T6*^{r} *met*$^-$ (P1), less than 1 count/min of radioactivity was detected in the 5MC region of the chromatogram when 300 counts/min were found in the 6MAP region. Hence, the amount of 5MC in the DNA of these strains is again less than 0.003 of the 6MAP. No other labeled methylated bases were found in any of the auxotrophs. Considering the similarities in the experiments with prototrophs and auxotrophs, the only distinguishing feature in the types and amounts of methylated bases in these strains is the difference in the 5MC content between derivatives of Bc251 and of C600.

Several observations support this conclusion. The thymine areas in the chromatograms of the hydrolysates of DNA were void of detectable radioactivity. Therefore, the values for 5MC in these strains have not been underestimated as a result of deamination. This measurement also ensured that the methylated bases found in preparations of DNA were not derived from any contaminant RNA, since thymine is the most abundant methylated base in the total RNA of *E. coli* and is present to the extent of 1% of the uracil in RNA (31).

To determine whether the difference in the 5MC content of derivatives of Bc251 and C600 was an artifact of preparation, strain Bc251 *T6*^{r} *met*$^-$ was grown in the presence of C^{14}-methyl-labeled methionine and strain C600 *T6*^{r} *met*$^-$

TABLE 2. *Methylated bases in the DNA of Escherichia coli**

Strain	Proportion (mole %) of bases in DNA present as		Ratio of 5MC-6MAP
	5MC	6MAP	
C600 *rm*$_4$ *T6*^{r} *met*$^-$ *ade*$^-$	0.11 ± 0.01	0.21 ± 0.02	0.57 ± 0.08
C600 *T6*^{r} *met*$^-$ *ade*$^-$	0.12 ± 0.01	0.22 ± 0.02	0.57 ± 0.08
Bc251 *T6*^{r} *met*$^-$	≤0.001	0.20 ± 0.04	≤0.003
Bc251 *T6*^{r} *met*$^-$ (P1)	≤0.001	0.22 ± 0.01	≤0.003

* DNA was extracted from cells grown in the presence of 1 to 2 μg/ml of H^3- or C^{14}-methyl-labeled methionine (3.2 to 4.7 counts per min per picomole) and hydrolyzed by PCA as described in Materials and Methods. Bases were separated by descending chromatography in solvent i. Bases from derivatives of Bc251 were further separated by ascending chromatography in a second dimension in solvent ii. Less than 1 count/min was found in the 5MC area of the chromatograms of bases from the DNA of derivatives of Bc251 under conditions of labeling which produced about 300 counts/min in the 6MAP region. The values given are averages of six determinations for C600 *T6*^{r} *met*$^-$ *ade*$^-$, five for C600 *rm*$_4$ *T6*^{r} *met*$^-$ *ade*$^-$, two for Bc251 *T6*^{r} *met*$^-$, and two for Bc251 *T6*^{r} *met*$^-$ (P1).

was grown in the presences of H^3-methyl-labeled methionine. The cultures were chilled, mixed, and harvested by centrifugation. The DNA extracted from the mixture was hydrolyzed with PCA and analyzed by chromatography in solvent i and also in solvent iii. The C^{14} radioactivity of strain Bc251 *T6*^{r} *met*$^-$ moved as a single spot with an R_F identical to that of reference 6MAP in both solvent systems. The 5MC regions contained less than 2% of the C^{14} radioactivity of the 6MAP areas. The H^3 radioactivity of strain C600 *T6*^{r} *met*$^-$ was partitioned between 5MC and 6MAP in the ratio of 0.6. Therefore, the absence of labeled 5MC in the DNA of strain Bc251 is a property of that DNA acquired before its purification, and is not a consequence of any 5-methylcytosine deaminase or demethylase activity which might be present in extracts of strain Bc251.

Methylated deoxyribosides in the DNA of E. coli. The DNA of C600 *T6*^{r} *met*$^-$, labeled with C^{14}-methyl-methionine, was hydrolyzed with purified snake venom phosphodiesterase and bacterial alkaline phosphatase. Chromatographic separation of the hydrolysate in solvent i revealed two major radioactive spots (Fig. 2). The slower spot corresponded to reference 5-methyldeoxycytidine (5MCdR). The other, faster spot had an R_F of 0.58, which compares with the R_F value of 0.59 reported for 6-methylaminopurine deoxyriboside (6MAPdr) by Dunn and Smith (16). Although further identification was not made, the ratio of the radioactivity in the slower component to that in the faster was 0.6, which agrees with the ratio for 5MC-6MAP found for the free bases after PCA hydrolysis. Of the radioactivity measured in the chromatogram, 89% was in the presumptive 5MCdR and 6MAPdR, 5% was at the origin, and the rest was diffusely distributed. No methylated deoxyribosides other

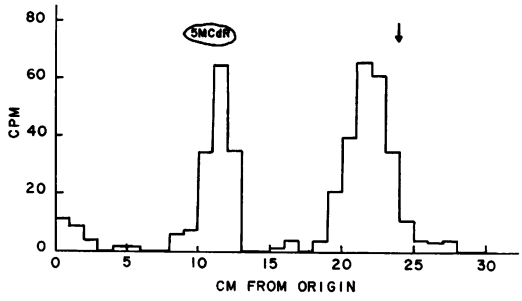

FIG. 2. *Chromatogram of methylated deoxyribosides from the DNA of Escherichia coli C600 T6*^{r}*met*$^-$. *DNA was extracted from cells grown in the presence of 2 μg/ml of C*^{14}*-methyl-labeled methionine (3.2 counts per min per picomole) and hydrolyzed by snake venom phosphodiesterase and bacterial alkaline phosphatase as described in Materials and Methods. The liberated deoxyribosides were separated by descending chromatography in solvent i. The enclosed area represents the region of authentic 5MCdR located by its absorption of ultraviolet light. The arrow indicates the R_F for 6MAPdR in this solvent reported by Dunn and Smith (16).*

than presumptive 5MCdR and 6MAPdR were detected. The methyl-labeled components of DNA revealed by enzymatic hydrolysis agree with those found by PCA hydrolysis, indicating that no methyl-labeled products were overlooked either as a result of destruction by acid or because of methylation of the sugar residue.

Methylated bases and deoxyribosides in the DNA of phage λ. The DNA of phage λ, labeled with C^{14}- or H^3-methyl-methionine, was hydrolyzed by PCA and analyzed by chromatography (Tables 3 and 4). The methylated bases present in phage λ were the same as those present in their host of origin, but the amounts and proportions differed

between λ and *E. coli*. A similar result was obtained when the methylated components were isolated by enzymatic procedures (Table 5). Again, for phage as for host cell, the DNA preparations with host specificity of strain K-12 and of strain C are not characterized by differences in methylated bases. The sole distinguishing feature in these DNA preparations is the absence of 5MC in DNA from Bc251 or from phage λ grown on that strain. This feature argues for the absence in Bc251 of an in vivo methylation of the 5-cytosine position in DNA with methyl groups derived from methionine, rather than a special sensitivity of 5MC groups in the DNA of Bc251 toward acid hydrolysis, since the results are similar for PCA and enzymatic hydrolyses of phage λ grown on Bc251. From the molecular weight of 3.4×10^7 Daltons (9) and the average weight of about 340 for a deoxyribonucleotide unit, a value of 10^5 nucleotides can be assigned to λ DNA.

TABLE 3. *Methylated bases in perchloric acid digests of the DNA of phage λ**

Phage	Radioactivity (count/min) in chromatogram regions		Amt (moles × 10^{-9}) of DNA hydrolyzed	Proportion of bases in DNA present as		Ratio of 5MC-6MAP	Residues per molecule of λ DNA	
	5MC	6MAP		5MC	6MAP		5MC	6MAP
λ·C600 *rm*$_4$ *T6*^{r} *met*$^-$ *ade*$^-$	84	194	27.5	0.05 ± 0.01	0.11 ± 0.01	0.43	50	110
λ·C600 *T6*^{r} *met*$^-$ *ade*$^-$	191	473	66.5	0.05 ± 0.01	0.11 ± 0.01	0.40	50	110

* Phage λ was grown in cells in the presence of 1 μg/ml of C^{14}-methyl-labeled methionine (6.3 counts per min per picomole). Measured amounts of the DNA from purified phage were hydrolyzed with PCA as described in Materials and Methods. Bases in the hydrolysates were separated by descending chromatography in solvent i. A value of 10^5 nucleotides per molecule of λ DNA was used to calculate the number of methylated bases present in λ.

TABLE 4. *Methylated bases in the DNA of phage λ carrying B host modification specificity**

Phage prepn	Radioactivity (count/min) in chromatogram regions		Amt (moles × 10^{-9}) of DNA hydrolyzed	Proportion of bases in DNA present as		Ratio of 5MC-6MAP	Residues per molecule of λ DNA	
	5MC	6MAP		5MC	9MAP		5MC	6MAP
1	≤0.5	635	112	≤0.00007	0.09 ± .01	≤0.001	≤0.1	90
2	≤0.6	431	64	≤0.00015	0.11 ± .01	≤0.002	≤0.2	110

* Phage λ was grown in cells of strain Bc251 *T6*^{r} *met*$^-$ in the presence of 1 μg per ml of C^{14}-methyl-labeled methionine (6.3 counts per min per picomole). Measured amounts of the DNA extracted from purified phage were hydrolyzed with PCA as described in Materials and Methods. Bases in the hydrolysate were separated by descending chromatography in solvent i followed by ascending chromatography in a second dimension in solvent ii. A value of 10^5 nucleotides per molecule of λ DNA was used for the calculation of the number of methylated bases present in λ.

TABLE 5. *Methylated bases in enzymatic digests of the DNA of phage λ**

Phage	Radioactivity (count/min) in chromatogram regions		Amt (moles × 10^{-9}) of DNA hydrolyzed	Proportion of deoxyribonucleosides in DNA present as		Ratio of 5MCdR-6MAPdR
	5MCdR	6MAPdR		5MCdR	6MAPdR	
λ·C600 *rm*$_4$ *T6*^{r} *met*$^-$ *ade*$^-$	99	258	23.2	0.04 ± 0.01	0.11 ± 0.01	0.39
λ·C600 *T6*^{r} *met*$^-$ *ade*$^-$	98	243	21.2	0.05 ± 0.01	0.11 ± 0.01	0.40
λ·Bc251 *T6*^{r} *met*$^-$	≤2.5	341	26.0	≤0.002	0.12 ± 0.01	≤0.002

* Phage λ was grown in cells in the presence of 1 μg/ml of H^3-methyl-labeled methionine (9.9 counts per min per picomole). Measured amounts of the DNA extracted from purified phage were hydrolyzed with snake venom phosphodiesterase and bacterial alkaline phosphatase as described in Materials and Methods. The liberated deoxyribonucleosides were separated by descending chromatography in solvent i.

After taking into account the possibility that side reactions may dilute the specific activity of the labeled methionine used in these experiments, the results in Table 4 indicate that there is less than one residue of 5MC per λ·Bc251 DNA molecule. However, in the absence of knowledge of the distribution of this trace 5MC among viable and nonviable phage, the possibility remains that a single residue of 5MC is relevant for the functioning of normal phage.

DISCUSSION

The bacteria chosen for the present study were two pairs of strains relatively isogenic except for differences in the modification they impart to DNA. *E. coli* C600 rm_4 is a mutant with C-type host modification specificity isolated from strain C600 which has K-12-type host modification specificity. *E. coli* Bc251 (P1) has P1-controlled modification in addition to the B-type host specificity present in its parent nonlysogenic strain.

All four bacterial strains contain similar amounts of 6MAP in their DNA. Strains C600 and C600 rm_4 have a similar content of 5MC in their DNA, but strains Bc251 and Bc251 (P1) lack this base or have only trace amounts. No correlation was found between methylated bases in DNA and the modification specificity which distinguishes K-12-type from C-type strains, or P1-type from non-P1-type strains. The DNA from C-type modificationless mutants of Bc251 and from K-12-type recombinants of crosses between K-12 and Bc251 is still void of 5MC (Lederberg, *in preparation*). Therefore, these modification specificities are not derived from the gross presence of that base. Ledinko (29) reported that the 5-methylcytosine deoxyribotide content of DNA was the same for λ grown on *E. coli* C600, C600 (P1), and C. Similarly, 5MC and 6MAP levels in the DNA of phage T1 could not be correlated with host modification specificity when such phage were produced without a disruption of host methionine metabolism (26). Clearly, if methylation of DNA is the basis for host modification, then its specificity must lie either in the arrangement and location of methylated residues or in only a minor fraction of these bases. In this connection, it is necessary, and perhaps sufficient, for some of the host-specific modifications to be at or near that end of the DNA molecule which first enters a restricting cell during bacterial mating or phage infection. If this localized protection can permit the functioning rather than the degradation of an entire DNA molecule, then it would be plausible that only a few nucleotide residues need be relevant. An examination of the distribution of methylated bases betwen the two strands and along the length of DNA of λ might assist in our understanding the function of these groups.

The DNA of phage λ contains the same methylated bases as its host of origin, but in different proportions. The DNA of coliphage T2 differs from the DNA of its host in its content of 6MAP (16). The quantitative differences among methylations of phage and bacterial DNA might be due to variations during infection in the synthesis or activity of the methyl tranferases, changes in the concentration of available *S*-adenosyl-L-methionine, or to a limited duration of accessibility of phage DNA to methylation before it acquires a protein coat. Since not all cytosine or adenine residues in DNA are methylated, some structural or compositional feature must differentiate the neighborhood of the methylatable site. Biases such as the preferential location of 5MC in pyrimidine isopliths (13) could also affect the ratio of 5MC to 6MAP in DNA.

By direct isolation of bases in DNA, Dunn and Smith (16) obtained values of about 1.8 moles of 6MAP per 100 moles of adenine or about 0.45 moles of 6MAP per 100 moles of bases in the DNA of strains B/R, K-12, and $15T^-$ of *E. coli*; 5MC was not reported. Using radiophosphorus label, Ledinko (29) obtained values of 0.07 to 0.08 mole per cent 5-methyldeoxycytidylate in the DNA of phage λ grown on strains W3110, C600, C600(P1), and C; 6MAP was not reported, nor was λ grown on strain B studied. The values reported here for the absolute amount of 6MAP and 5MC in the DNA of phage λ and its hosts may be lower limits, because it is possible that some methyl groups were made available from sources other than methionine, or from recycling of the *S*-adenosyl-L-homocysteine formed in transmethylation. Several enterobacterial strains can degrade *S*-adenosyl-L-homocysteine to ribosyl-L-homocysteine (14, 15). If this product is further metabolized to homocysteine, then two separate pathways are known which carry out the transfer of a methyl group from 5-methyltetrahydropteroylglutamate or the triglutamate to form methionine (19, 22, 23). Any methionine synthesized in this fashion would dilute the labeled methionine added to the medium. The extent of this recycling would be expected to depend on whether these interconversions are repressed or inhibited under a given set of physiological conditions. These considerations may apply to the labeling of methylated bases in the nucleic acids of *E. coli* strain 58-161 (42) whose methionine requirement is satisfied by homocysteine (39). However, these possibilities would not

affect the present conclusions on the role of methionine-derived methyl groups, since strain Bc251 and its P1-lysogen share the same *met*$^+$ or *met*$^-$ alleles, and *met*$^-$ mutants of strains C600 and C600 *rm*$_4$, whose methionine requirements are satisfied by homocysteine and vitamin B_{12}, respectively, yielded similar results to each other and to their *met*$^+$ parents.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Predoctoral Fellowship 5-F1-GM-21, 041-02(MG), Public Health Training Grant HD-00019-05, and National Science Foundation Grant GB-1945.

LITERATURE CITED

1. APPLEYARD, R. K. 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
2. ARBER, W. 1965. Host specificity of DNA produced by *Escherichia coli*. V. The role of methionine in the production of host specificity. J. Mol. Biol. **11**:247–256.
3. ARBER, W., AND D. DUSSOIX. 1962. Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ. J. Mol. Biol. **5**:18–36.
4. ARBER, W., AND C. LATASTE-DOROLLE. 1961. Erweiterung des Wirtsbereiches des Bakteriophagen λ auf *Escherichia coli*. Pathol. Mikrobiol. **24**:1012–1018.
5. ARBER, W., AND M. L. MORSE. 1965. Host specificity of DNA produced by *Escherichia coli*. VI. Effect on bacterial conjugation. Genetics **51**: 137–148.
6. BERTANI, G., AND J. J. WEIGLE. 1953. Host controlled variation in bacterial viruses. J. Bacteriol. **65**:113–121.
7. BOICE, L. B., AND S. E. LURIA. 1963. Behavior of prophage P1 in bacterial matings. I. Transfer of the defective prophage P1*dl*. Virology **20**: 147–157.
8. BOYER, H. 1964. Genetic control of restriction and modification in *Escherichia coli*. J. Bacteriol. **88**:1652–1660.
9. BURGI, E., AND A. D. HERSHEY. 1963. Sedimentation rate as a measure of molecular weight of DNA. Biophys. J. **3**:309–321.
10. BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**:315–323.
11. CANTONI, G. L. 1953. S-adenosylmethionine; a new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. J. Biol. Chem. **204**:403–416.
11*a*. COHEN, D. 1959. A variant of phage *P2* originating in *Escherichia coli* strain *B*. Virology **7**:112–126.
12. DOSKOČIL, J., AND Z. ŠORMOVÁ. 1965. The occurrence of 5-methylcytosine in bacterial deoxyribonucleic acids. Biochim. Biophys. Acta **95**:513–515.
13. DOSKOČIL, J., AND Z. ŠORMOVÁ. 1965. The sequences of 5-methylcytosine in the DNA of *Escherichia coli* Biochem. Biophys. Res. Commun. **20**:334–339.
14. DUERRE, J. A. 1962. A hydrolytic nucleosidase acting on S-adenosylhomocysteine and on 5′-methylthioadenosine. J. Biol. Chem. **237**: 3737–3741.
15. DUERRE, J. A., AND P. M. BOWDEN. 1964. Utilization of ribosylhomocysteine by various microorganisms. Biochem. Biophys. Res. Commun. **16**:150–155.
16. DUNN, D. B., AND J. D. SMITH. 1958. The occurrence of 6-methylaminopurine in deoxyribonucleic acids. Biochem. J. **68**:627–636.
17. DUSSOIX, D., AND W. ARBER. 1965. Host specificity of DNA produced by *Escherichia coli*. IV. Host specificity of infectious DNA from bacteriophage lambda. J. Mol. Biol. **11**:238–246.
18. EGAMI, F., H. ISHIHARA, AND M. SHIMOMURA. 1953. Über die methylierte Heferibonucleinsäure. Z. Physiol. Chem. **295**:349–354.
19. FOSTER, M. A., G. TEJERINA, AND D. D. WOODS. 1961. Two enzymic mechanisms for the methylation of homocysteine by *Escherichia coli*. Biochem. J. **81**:1p
20. GAREN, A., AND C. LEVINTHAL. 1960. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of E. coli. I. Purification and characterization of alkaline phosphatase. Biochim. Biophys. Acta **38**:470-483.
21. GOLD, M., AND J. HURWITZ. 1963. The enzymatic methylation of the nucleic acids. Cold Spring Harbor Symp. Quant. Biol. **28**:149–156.
22. GUEST, J. R., S. FRIEDMAN, AND M. A. FOSTER. 1962. Alternative pathways for the methylation of homocysteine by *Escherichia coli*. Biochem. J. **84**:93p.
23. HATCH, F. T., A. R. LARRABEE, R. E. CATHOU, AND J. H. BUCHANAN. 1961. Enzymatic synthesis of the methyl group of methionine. I. Identification of the enzymes and co-factors involved in the system isolated from *Escherichia coli*. J. Biol. Chem. **236**:1095–1101.
24. KAISER, A. D., AND D. S. HOGNESS. 1960. The transformation of *Escherichia coli* with deoxyribonucleic acid isolated from bacteriophage λ *dg*. J. Mol. Biol. **2**:392–415.
25. KELLER, E. B. 1964. The hydrolysis of "soluble" ribonucleic acid by snake venom phosphodiesterase. Biochem. Biophys. Res. Commun. **17**:412–415.
26. KLEIN, A., AND W. SAUERBIER. 1965. Role of methylation in host controlled modification of phage T1. Biochem. Biophys. Res. Commun. **18**:440–445.
27. LEDERBERG, S. 1957. Suppression of the multiplication of heterologous bacteriophage in lysogenic bacteria. Virology **3**:496–513.
28. LEDERBERG, S. 1965. Host-controlled restriction and modification of deoxyribonucleic acid in *Escherichia coli*. Virology **27**:378–387.

29. LEDINKO, N. 1964. Occurrence of 5-methyldeoxycytidylate in the DNA of phage λ. J. Mol. Biol. **9**:834–835.
30. LENNOX, E. S. 1955. Transduction of linked genetic characters of host by bacteriophage P1. Virology **1**:190–206.
31. LITTLEFIELD, J. W., AND D. B. DUNN. 1958. The occurrence and distribution of thymine and three methylated-adenine bases in ribonucleic acids from several sources. Biochem. J. **70**:642–651.
32. LURIA, S. E. 1953. Host-induced modifications of viruses. Cold Spring Harbor Symp. Quant. Biol. **18**:237–244.
33. MANDELL, J., AND J. GREENBERG. 1960. A new chemical mutagen for bacteria, 1-methyl-3-nitro-1-nitrososguanidine. Biochem. Biophys. Res. Commun. **3**:575–577.
34. MARKHAM, R., AND J. D. SMITH. 1949. Chromatographic studies of nucleic acids. I. A technique for the identification and estimation of purine and pyrimidine bases, nucleosides, and related substances. Biochem. J. **45**:294–298.
35. MARSHAK, A., AND H. J. VOGEL. 1951. Microdetermination of purines and pyrimidines in biological materials. J. Biol. Chem. **189**:597–605.
36. MESELSON, M. 1964. On the mechanism of genetic recombination between DNA molecules. J. Mol. Biol. **9**:734–745.
37. PITTARD, J. 1964. Effect of phage-controlled restriction on genetic linkage in bacterial crosses. J. Bacteriol. **87**:1256–1257.
38. SAITO, H., AND K. I. MIURMA. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim. Biophys. Acta **72**:619–629.
39. SIMMONDS, S. 1948. Utilization of sulfur-containing amino acids by mutant strains of *Escherichia coli*. J. Biol. Chem. **174**:717–722.
40. SUSSMAN, R., AND F. JACOB. 1962. Sur un système de répression thermosensible chez le bactériophage. λ d'*Escherichia coli*. Compt. Rend. **254**:1517–1519.
41. SZER, W., AND D. SHUGAR. 1960. N-methylation of uridylic acid and preparation of oligonucleotides of 3-methyluridylic acid. Acta Biochim. Polon. **7**:491–504.
42. TATUM, E. L. X-ray induced mutant strains of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **31**:215–219.
43. WEIGLE, J., M. MESELSON, AND K. PAIGEN. 1959. Density alterations associated with transducing ability in bacteriophage lambda. J. Mol. Biol. **1**:379–386.
44. WILLIAMS, E. J., S-c. SUNG, AND M. LASKOWSKI, SR. 1961. Action of venom phosphodiesterase on deoxyribonucleic acid. J. Biol. Chem. **236**: 1130–1134.
45. WYATT, G. R. 1951. Recognition and estimation of 5-methylcytosine in nucleic acids. Biochem. J. **48**:581–584.