ROLE OF METHYLATION IN HOST CONTROLLED MODIFICATION OF PHAGE T1

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Non-mutational changes upon growth on certain bacterial host strains concerning host range properties have been found in many bacteriophages (see e.g. Bertani, 1958). This phenomenon is known as host controlled modification (HCM). Phage particles thus carry a host specificity determined by the bacterial strain on which they were produced. Upon infection of a different bacterial strain the phage may be either accepted or rejected on the basis of its specificity. If accepted, it multiplies in the new host cell and acquires a new specificity. Arber and Dussoix (1962) and Dussoix and Arber (1962) showed that host specificity of phage λ is carried by the phage DNA. The chemical basis of HCM in λ is unknown. It was suggested by in vitro experiments of Gold and Hurwitz (1963) that different methylation of λ DNA by different host cells might confer different host specificity to the phage. Ledinko (1964), however, found equal amounts of 5-methylcytosine (5-MC) in DNA extracted from phage λ carrying the host specificities derived from the strains Escherichia coli C, B, K, or K(P1).

Evidence will be presented here that (1) T1-DNA may in some cases be methylated to different extents depending on its host specificity and (2) that methylation is the chemical basis or at least an absolute requirement for HCM of phage T1 by bacterial host cells lysogenic for P1. In addition a new system of HCM acting on T1 will be described which does not overlap with the P1 system.

Material

Bacteria: E. coli strains Bc 251, referred to as Bc; Bc(P1) carrying prophage P1; B 94 auxotrophic for adenine; B 94(P1); Hfr U U^-, a K 12 strain requiring uracil and vitamin B1; Hfr U U^-(P1); Hfr U/3 U^- resistant to phage T3; Hfr U G^- deficient for guanine; and Hfr U G^-(P1); Shigella Sh(P1); and E. coli 15 THU, a strain auxotrophic for thymine, histidine, and uracil, re-

ferred to as THU. Growth media were M-9 supplemented with 0.3% Difco vitamin free casamino acids, other supplements being mentioned in the text, and LB medium (Bertani, 1951). Descending paper chromatography was performed using Whatman No. 1 paper and the following solvent systems. 1) Butanol/water, 86:14; 2) isopropanol/HCl/water, 170:41:39; 3) 5% aqueous Na_2HPO_4 saturated with isoamyl alcohol. The reference substances 5-methylcytosine (5-MC) and 6-methylaminopurine (6-MAP) used in chromatography were purchased from Calbiochem.

Experiments and results

Lederberg (1957) showed that bacterial strains lysogenic for P1 produce HCM in T1, T3, and other phages. Glover et al. (1963) demonstrated that both restriction and modification of the growing phage is controlled by P1 prophage. To see whether different host specificities are paralleled by different degrees of methylation of T1 DNA we used phage modified (1) in the P1 HCM system mentioned above and (2) in a newly discovered HCM system with THU host cells. Table I shows the plating efficiencies of T1 of different host specificities.

Table I Plating efficiencies of T1, depending on its host specificity, on different bacterial host strains

	Bc, Hfr U	Bc(P1), Hfr U(P1), Sh(P1)	THU
T1.Bc, T1.Hfr U	1	10^{-4}-10^{-5}	10^{-2}
T1.Bc(P1), T1.Hfr U(P1)	1	1	10^{-2}
T1.THU	1	10^{-4}-10^{-5}	0.85

It is seen that T1 grown on nonlysogenic strains is restricted in strains lysogenic for P1 but shows full plating efficiency once having grown on such a strain. The same is true, when T1 - grown on E. coli Bc, K, or Shigella Sh irrespective of their lysogenicity for P1 - infects a THU cell.

DNA from phage grown in nonlysogenic K and B strains, on P1-lysogenic host bacteria, and in THU was analysed for methylated bases. For this purpose phage DNA was labeled with the desired tritiated bases and examined for the methylated derivative(s) after hydrolysis of the phage DNA, or in some cases total phage.

Host cells for lysates were grown in M-9, supplemented by the necessary substances to allow growth of the auxotrophic

bacteria, to a concentration of 2 x 10^8/ml, washed with phosphate buffer, resuspended in growth medium deficient in the base which was to be labeled in the phage DNA, and aerated for 40-60 minutes. Growth temperatures were 28° with P1-lysogenic strains and 37° with the others. Cells were then collected by centrifugation and resuspended in T1 adsorption medium (Watson, ref. Benzer, 1952) with M/500 KCN at growth temperatures to a concentration of 10^9/ml. T1 was then added at a multiplicity of infection (m.o.i.) of 3-5. Adsorption was better than 90% after 30 minutes. The infected cells were then collected by centrifugation and resuspended in M-9 containing each DNA base not to be labeled as its riboside at a concentration of 30 µg/ml, the base to be labeled at a concentration of 20 µg/ml in the case of cytosine, or 30 µg/ml in the case of adenine. Usually lysates with titers of 3-6 x 10^{10} were obtained. Phage purification involved concentration of the lysates at 30° in a flash evaporator, sedimentation in an ultracentrifuge, followed by digestion with 15 µg/ml each of DNase and RNase at room temperature for 2 hours. The phages were then sedimented twice in the ultracentrifuge and banded in a CsCl density gradient. DNA was extracted by the phenol method and phenol removed by dialysis. DNA, or in some cases total phage, was hydrolysed with formic acid (Wyatt and Cohen, 1953). Bases were separated by descending paper chromatography. To purify methylcytosine from cytosine contamination, it was run in solvent system 2 and had to be rechromatographed in solvent system 1 for three times. Hydrolysates of ^{3}H-adenine phage were run in solvent system 1 and methyladenine (6-MAP) rechromatographed in solvent system 3. Paper strips were cut into 1 cm broad pieces and radioactivity determined by liquid scintillation counting. Radioactive 5-MC and 6-MAP from hydrolysates were identified by simultaneous chromatography of reference substances on the same strip. The results are shown in Table II.

If methylation is involved in P1 HCM of T1 DNA, these data suggest that only the pattern of methylation is changed or that quantitative differences in methylated cytosine and adenine are too small to be detected. In the THU system cytosine in T1 DNA is less frequently methylated upon growth in the restrictive host cell. We were unable to find methylated derivatives of guanine.

Table II Methylated bases from T1 DNAs of different host specificities. Values are given in mole per cent of the nonmethylated bases.

	5-MC+	6-MAP
T1.THU	0.16	-
T1.Hfr U U⁻	0.28	-
T1.Hfr U U⁻(P1)	0.26	-
T1.B 94	-	2.1
T1.B 94(P1)	-	2.1

+Values for 5-MC are corrected for the fact that 5,6-^{3}H-cytosine was used in the experiments. Correction was based on the assumption of equal label in both positions, which is not sure. Relative values are not impaired, since tritiated cytosine from the same batch was used throughout.

A means to test the importance of methylation for HCM would be to inhibit cells from methylating phage DNA. In contrast to Lederberg (1957) Schell et al. (1963) found that T3 does not grow on K(P1) strains. The same is true for Bc(P1). Gold et al. (1964) discovered that T3 infecting E. coli cells induces an enzyme which cleaves S-adenosyl-methionine in vitro. They had previously found (Gold et al., 1963) that this substance is the only substrate for DNA-methylating enzymes and therefore suggested that T3 DNA cannot be methylated in the cells and is therefore susceptible to the restricting principle in P1 lysogenic cells. If methylation were the mechanism of T1 modification by prophage P1, simultaneous infection with T1 and T3 of those rare P1 lysogenic cells which accept T1 and allow the function of the T3 genome should result in a great deal of unmodified T1 phage in the progeny. In order to test the influence of T3 coinfection on T1 methylation a lysate was made from mixedly infected B 94 cells, (m.o.i. T1 = 5; T3 = 5), labeling adenine as described above. T3 was removed to 1 particle per 1000 T1 by adsorption to B/1 cells. T1 DNA was then analysed for adenine and 6-MAP. It contained 1% 6-MAP, which is less than 50% of the value found in T1 grown without T3.

To test whether the modification of T1 particles is impaired, as

well, when grown in Bc(P1) cells simultaneously infected with T3, the following experiment was performed: Bc(P1) cells were grown overnight, 1:10 diluted into fresh medium, and aerated for 100 minutes. They were collected by centrifugation and resuspended in T1 adsorption medium at a concentration of 6×10^8/ml. M.o.i. was 0.8 for T1 and 7 for T3. Adsorption was better than 95% under the conditions mentioned above. The infected cells were washed twice. T1-antiserum was then added 1:10 and the mixture incubated for 15 minutes at 28° whereafter the cells were once more washed, which removed free T1 completely. Cells were diluted and infective centers scored on Sh(P1) and on Hfr U/3, respectively, using the agar layer technique (Adams, 1959). The results are shown in table III.

Table III Plating efficiencies of T1 and of T1+T3 infected P1 lysogenic cells on lysogenic and nonlysogenic bacteria. Data are given in per cent of plaque forming cells, 100% being about 10^{-3}, which is the accepting fraction of the infected cells.

	Sh(P1)	Hfr U/3
Bc(P1)+T1	100	100
Bc(P1)+T1+T3	1	20

It can be seen that only 5% of the cells yielding T1 phage after infection with T1+T3 produce modified phage. This result strongly suggests that HCM of T1 DNA by lysogenic host bacteria involves methylation of the DNA, which can be suppressed by simultaneous infection with T3.

While T3 does not grow on P1 lysogenic strains, it does grow on THU with 5% plating efficiency. Coinfection experiments with T1 and T3 in THU showed quite different results from those presented in table III. T3 did not influence the T1 modifying capacity of the accepting cells. This indicates that either HCM of T1 by THU does not involve any methylation, or that methyl groups for DNA methylation are available in sufficient amount for T1 modification even in T3 coinfected THU cells.

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