JOURNAL OF VIROLOGY, May 1971, p. 690-691

Vol. 7, No. 5
Printed in U.S.A.

Variation of 6-Methylaminopurine Content in Bacteriophage P22 Deoxyribonucleic Acid as a Function of Host Specificity

STANLEY HATTMAN

Department of Biology, University of Rochester, Rochester, New York 14627

Received for publication 25 January 1971

The 6-methylaminopurine (MAP) content of P22 deoxyribonucleic acid has been analyzed as a function of the host specificity it carries. A 40 to 50% reduction in MAP level occurs as a result of growth in host cells defective in the ability to confer LT specificity.

Host-induced modification has been described in a variety of phage-bacterial systems (see reviews 3, 11, 16). The phenomeon is characterized by a nonheritable (phenotypic) alteration in the host range (host specificity) of the virus produced as a result of a single cycle of growth in a particular host. There is good evidence that specific methylation of adenine bases plays a role in determining the host specificity of phage deoxyribonucleic acid (DNA; references 2, 9, 12). Despite the indirect evidence indicating that DNA methylation determines the various phage λ host specificities (1, 10), direct attempts to correlate these with gross changes in the level of methylated bases have been unsuccessful (8, 13, 14).

It was of interest, therefore, to consider this question with *Salmonella* phage P22. This system is particularly useful inasmuch as it is known that the phage DNA can contain 6-methylaminopurine (MAP; reference 6) and because a variety of host strains are available which possess altered modification-restriction properties (5, 7, 15, 19, 21). The results of a partial comparative analysis of P22 DNA MAP contents as a function of host specificity are reported.

The strains of *S. typhimurium* used in this study are listed in Table 1.

P22 was grown in medium containing adenine-2-^3H by the procedure described previously (9). The labeled phage was purified by alternate cycles of high- and low-speed centrifugation and by sedimentation-equilibrium centrifugation in a CsCl gradient. Isolation of the phage DNA and determination of its MAP content were as described earlier (9).

The results of an analysis of a series of independent phage preparations are listed in Table 2. The chromatographic distribution of the labeled purine bases was similar to that reported for T-even phage (9) and is not illustrated here. The salient findings are summarized as follows. (i) P22 grown on a variety of strains conferring LT specificity (m_{LT}^+) have roughly the same MAP content (0.49 to 0.55 mole % of adenine). (ii) P22 grown on strains unable to confer LT specificity (m_{LT}^-) contains 40 to 50% less MAP. (iii) The presence of modifying (N-3) or nonmodifying R factors (21) has little influence on the overal P22 MAP level (in m_{LT}^+) hosts).

Thus, in contrast to the λ modification system, changes in LT specificity are correlated with 40 to 50% changes in P22 DNA MAP. This result appears directly related to the modification defect (m_{LT}^-) and not to the restriction defect (r_{LT}^-), since the r_{LT}^- m_{LT}^+ strain methylated P22 DNA to roughly the same extent as did the r_{LT}^+ m_{LT}^+ strain (Table 2).

Since P22 DNA has a molecular weight of 27×10^6 daltons (17) and contains ca. 26% adenine bases (18, 20), 0.50 mole % MAP corresponds to approximately 100 residues per DNA molecule. Thus, loss of the LT host specficity parallels a loss of approximately 40 to 50 MAP from the chromosome. We have no idea what proportion (if any) of these are essential for LT specificity or whether the remaining MAP is located at different sites on the DNA and, consequently, causes loss of LT specificity. It seems clear though that a different MAP content or arrangement is essential for the virus to carry proper specificity in its DNA.

A number of important questions still remain open. For example, does P22 contain other methylated bases (e.g., 5-methylcytosine) in addition to MAP and what possible role may they

play in host specificity? Lastly, we do not know whether P22 induces its own DNA methylase and, if so, how much MAP (or 5-methylcytosine) may be contributed by this activity.

TABLE 1. *Strains of Salmonella typhimurium and relevant properties*

Strain[a]	Host specificity[b] phenotype
LT2 wild type	$r^+_{LT}m^+_{LT}$
LT2 (222)	$r^+_{LT}m^+_{LT}$
LT2 (N − 1)	$r^+_{LT}m^+_{LT}$
LT2 (N − 3)	$r^+_{LT}m^+_{LT}r^+_{N-3}m^+_{N-3}$
LT2 proC90 *fer*[c]	$r^-_{LT}m^+_{LT}$
LT2 proC90 *fer*	$r^-_{LT}m^-_{LT}$
LT7 proB215 Sm^r *fer*	$r^-_{LT}m^-_{LT}$
LT7 leu119 Sm^r *fer*	$r^-_{LT}m^-_{LT}$

[a] Strains containing R factor (N − 1, N − 3, 222) were provided by D. Botstein; LT2 wild type was from K. Sanderson; LT2 proC90 strains were produced by C. Colson and obtained from H. Boyer; LT7 strains were from P. Hartman via R. Benzinger. Further reference to host strains will be simply denoted LT and not LT2 or LT7.

[b] The terminology for the host specificity phenotype is that proposed earlier (5). Thus, r_{LT} and m_{LT} refer to LT-specific restriction and modification, respectively.

[c] The *fer* mutation indicates a loss of restriction towards *E. coli* DNA (4, 15).

TABLE 2. *6-Methylaminopurine (MAP) content of DNA derived from P22 grown on various hosts*

Phage[a]	Mole % MAP[b]
P22·$r^+_{LT}m^+_{LT}$	0.56 (0.54, 0.61, 0.49, 0.60)
P22·$r^+_{LT}m^+_{LT}$(N−1)	0.56 (0.57, 0.60, 0.50, 0.56)
P22·$r^+_{LT}m^+_{LT}$(N−3)	0.51 (0.48, 0.47, 0.57)
P22·$r^+_{LT}m^+_{LT}$(222)	0.57 (0.58, 0.55)
P22·$r^-_{LT}m^+_{LT}$[c]	0.49 (0.46, 0.52)
P22·$r^-_{LT}m^-_{LT}$[c]	0.27 (0.28, 0.27)
P22·$r^-_{LT}m^-_{LT}$[d]	0.32; 0.33

[a] A spontaneous clear plaque-forming mutant was derived from P22 wild type and used in all the experiments described.

[b] Mole % MAP = counts per min of MAP/(counts per min of MAP + counts per min of adenine) × 100. Values in parenthesis were obtained from independent preparations and chromatographic analysis.

[c] Derived from proC90 strain (Table 1).

[d] Derived from LT7 leu 119 and LT7 proB215, respectively (Table 1).

This investigation was supported by Public Health Service grant AI-083738 from the National Institute of Allergy and Infectious Diseases. The MAP determinations for P22 grown on LT7 $r^-_{LT}m^-_{LT}$ were ably performed by Alan Plotnik.

LITERATURE CITED

1. 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.
2. Arber, W., and U. Kühnlein. 1967. Mutationeller Verlust B-spezifischer Restriktion des Bakteriophagen *fd*. Pathol. Microbiol. 30:946–952.
3. Arber, W., and S. Linn. 1969. DNA modification and restriction. Annu. Rev. Biochem. 38:467–500.
4. Colson, C., and A. M. Colson. 1967. Host specificity and fertility in *Salmonella typhimurium* LT7. Biochem. Biophys. Res. Commun. 29:692–695.
5. Colson, A. M., C. Colson, and A. van Pel. 1969. Host-controlled restriction mutants of *Salmonella typhimurium*. J. Gen. Microbiol. 58:57–64.
6. Dunn, D. B., and J. D. Smith. 1958. The occurrence of 6-methylaminopurine in deoxyribonucleic acids. Biochem. J. 68:627–636.
7. Garen, A., and N. D. Zinder. 1955. Radiological evidence for the partial genetic homology between bacteriophage and host bacteria. Virology 1:347–376.
8. Gough, M., and S. Lederberg. 1966. Methylated bases in the host-modified deoxyribonucleic acid of *Escherichia coli* and bacteriophage λ. J. Bacteriol. 91:1460–1468.
9. Hattman, S. 1970. DNA methylation of T-even bacteriophages and of their nonglucosylated mutants: its role in P1-directed restriction. Virology 42:359–367.
10. Hirsch-Kauffmann, M., and W. Sauerbier. 1968. Inhibition of modification and restriction for phages λ and T1 by co-infecting T3. Mol. Gen. Genet. 102:89–94.
11. Klein, A. 1965. Wirtskontrollierte Modifikation. Z. Vererbungslehre 96:324–345.
12. Klein, A. 1965. Mechanismen der Wirtskontrollierten Modifikation des Phagen T1. Z. Vererbungslehre 96:346–363.
13. Lederberg, S. 1966. 5-Methylcytosine in the host-modified DNA of *Escherichia coli* and phage λ. J. Mol. Biol. 17:293–297.
14. Ledinko, N. 1964. Occurrence of 5-methyldeoxycytidylate in the DNA of phage lambda. J. Mol. Biol. 9:834–835.
15. Okada, M., T. Watanabe, and T. Miyake. 1968. On the nature of the recipient ability of *Salmonella typhimurium* for foreign deoxyribonucleic acids. J. Gen. Microbiol. 50:241–252.
16. Revel, H. R., and S. E. Luria. 1970. DNA-glucosylation in T-even phage: genetic determination and role in phage-host interaction. Annu. Rev. Genet. 4:177–192.
17. Thomas, Jr., C. A. 1966. The arrangement of information in DNA molecules. J. Gen. Physiol. 49:143–169.
18. Thomas Jr., C. A., and T. C. Pinkerton. 1962. Sedimentation equilibrium studies on intact and fragmented bacteriophage DNA. J. Mol. Biol. 5:356–372.
19. Uetake, H., S. Toyama, and S. Hagirawa. 1964. On the mechanism of host-induced modification. Multiplicity activation and thermolabile factor responsible for phage growth restriction. Virology 22:202–213.
20. Volkin, E., L. Astrachan, and J. Countryman. 1952. Metabolism of RNA phosphorous in *Escherichia coli* infected with bacteriophage T7. Virology 6:545–555.
21. Watanabe, T., T. Takano, T. Arai, H. Nishida, and S. Sato. 1966. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. X. Restriction and modification of phages by *fi*$^-$ R factors. J. Bacteriol. 92:477–486.