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Host-Controlled Modification and Restriction of Bacteriophage T7 by *Escherichia coli* B

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T7 phage resists *Escherichia coli* B host-controlled modification and restriction in vivo, but its DNA carries roughly five sites which are susceptible to the purified enzymes.

When DNA is replicated in *Escherichia coli* B it is normally "modified" through the methylation of specific sites, thus rendering it resistant both to the B-restriction endonuclease in vivo or in vitro and to further methylation by the B-modification enzyme in vitro (1, 2, 7). We report here that phage T7 DNA, while neither restricted nor modified in vivo, is a substrate for both activities in vitro.

Bacterial and phage strains are as reported previously (7), except for *E. coli* B/1 and CW-6. (*E. coli* B/1 is a B strain made resistant to phage T1 obtained from M. Chamberlin; *E. coli* CW-6 is a derivative of B/r into which the K-12 *hsp* loci were introduced by conjugation in the laboratory of H. Boyer and D. Roulland-Dussoix, and which was made *his*$^-$*thy*$^-$ by C. Ward.) T7 DNA was prepared by phenol extraction, and fd replicative form DNA (RF) was prepared as previously described (7).

T7 DNA grown in hosts carrying either the B or K *hsp* loci is a substrate for purified B-restriction endonuclease, being degraded to fragments with an average size of 5.4×10^6 daltons (Fig. 1). The degradation is dependent upon the presence in the reaction mixture of S-adenosylmethionine and ATP. In addition, the B-modification methylase imparts roughly eight methyl groups to either type of DNA molecule (Table 1). The average size of the restricted DNA and the number of methyl groups transferred (corrected for the level of incomplete methylation observed with the fd RF) imply that the T7 DNA has roughly five specificity sites.

These results demonstrate that *E. coli* B/1 does not modify the T7 DNA. However, a partially purified extract from *E. coli* B/1, but not one from CW-6, contains B-modification activity, since it can protect fd RF from restriction by *E. coli* B spheroplasts (Table 2). The B/1 extract, but not the CW-6 extract, also contains restriction enzyme, as evidenced by an activity dependent on S-adenosylmethionine (a cofactor unique to the restriction endonuclease (2)) that converts fd RF to a linear form which is subsequently hydrolyzed by exonuclease in the extract (Fig. 2). However, as evidenced by the efficiency of plating of T7 on B/1 *versus* that on CW-6, there is no in vivo restriction of phage T7 by *E. coli* B/1 (Table 3), confirming an earlier report (3).

Several other phage are not subject to host-controlled modification and restriction in *E. coli*

TABLE 1. *Methylation of T7 and fd RF DNA*[a]

DNA substrate[b]	Methyl groups incorporated (pmol)	Estimated methyl groups incorporated per DNA molecule[c]	Estimated no. of sites[c]
fd RF·B, 1.5 nmol	<0.01	0	
fd RF·O, 1.5 nmol	0.35	3.1	≡2
T7·B/1, 2.9 nmol	0.28	7.5	4-5
T7·CW-6, 1.7 nmol	0.18	8.1	4-5

[a] Reactions were for 150 min with 0.75 μg of pure B-modification enzyme (an excess) and S-adenosyl-[*methyl*-^{3}H]methionine (9,300 counts per min per pmol) as previously described (7).

[b] RF·B and RF·O refer to RF isolated from a B-modifying and a nonmodifying host, respectively. Similarly, T7·B/1 and T7·CW-6 were grown on *E. coli* B/1 and CW-6.

[c] The number of nucleotides assumed for T7 DNA and fd RF were 76,400 (4) and 13,200 (8), respectively. We assume that each site can accept 2 methyl groups (1), although the in vitro reaction generally shows incorporation of roughly 1.5 to 1.7 per site (7).

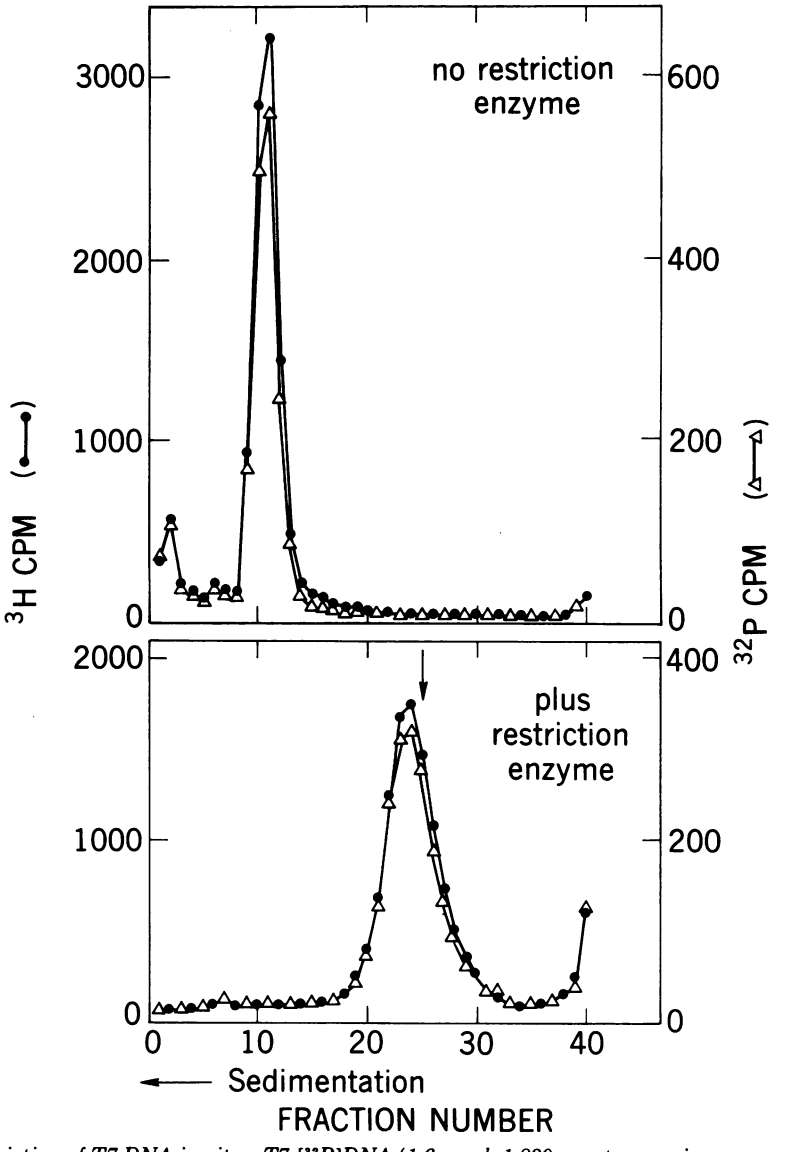

FIG. 1. *Restriction of T7 DNA in vitro. T7 [^{32}P]DNA (1.6 nmol, 1,830 counts per min per nmol) grown on E. coli B/1, and T7 [^{3}H]DNA (1.5 nmol, 11,200 counts per min per nmol) grown on E. coli CW-6 were incubated together in 140 μliters for 15 min at 37 C with 4 units of purified restriction enzyme as previously described (2). The DNA was then centrifuged through 5 to 20% sucrose gradients (5.2 ml) for 125 min at 65,000 rpm in the Spinco SW65L rotor, and collected and counted as described previously (2). The arrow shows the position of fd RF after being converted to a linear form by the restriction enzyme. Assuming that the molecular weight of T7 DNA and the RF are 25.2 × 10^6 (4), and 4.4 × 10^6 (8), respectively, the average molecular weight of the restricted T7 DNA was calculated (4) to be 5.4 ± 0.6 × 10^6.*

B (3). Phage T2, T4, and T6 are resistant, presumably because their DNA contains hydroxymethylcytosine, and T3 is resistant because an enzyme is induced which cleaves S-adenosylmethionine (5). However, T7 DNA does not contain hydroxymethylcytosine and the phage does not induce an S-adenosylmethionine-cleaving enzyme. Indeed, its DNA contains methylated nucleotides (5). Other nonrestricted phages, such as φX-174, do not possess *E. coli* B recognition sites. However, the results presented here demonstrate that T7 DNA does

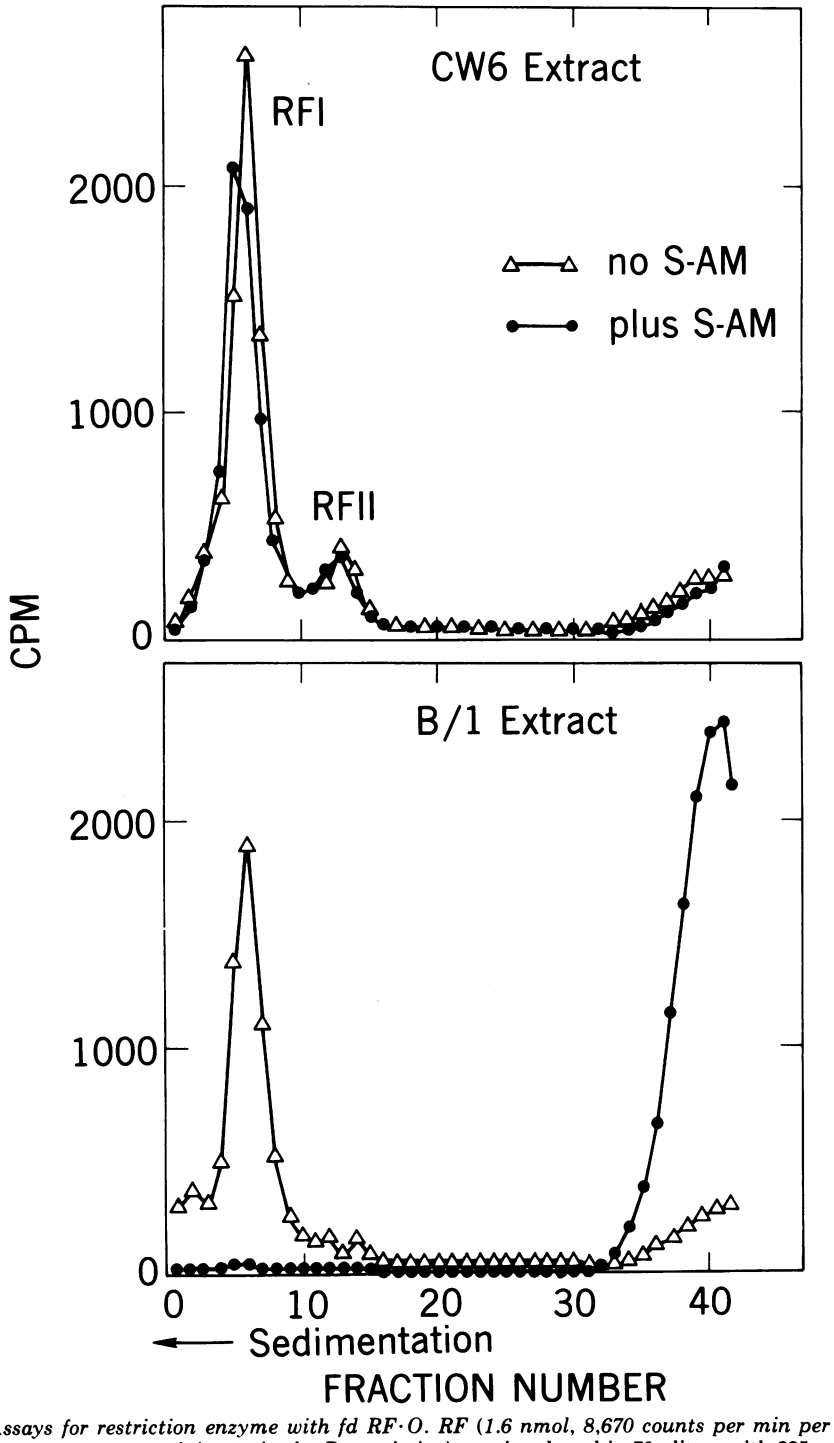

FIG. 2. *Assays for restriction enzyme with fd RF·O. RF (1.6 nmol, 8,670 counts per min per nmol) from strain fd 101 (a mutant containing 1 site for B-restriction) was incubated in 70 μliters with 225 μg of dialyzed ammonium sulfate fraction and S-adenosylmethionine (S-AM), as indicated, under conditions previously described (2). The DNA was then centrifuged through 5 to 20% sucrose gradients (5.2 ml) for 4.5 h at 50,000 rpm in the Spinco SW50.1 rotor. RFI is a covalently-closed duplex circular molecule and RFII is a duplex circular molecule with one or more single strand breaks.*

TABLE 2. *Assays for modification enzyme with fd RF·O*[a]

Enzyme	PFU/OD_{260} DNA × 10^{-8}[b]		Normalized ratio[c]	Fraction of sites modified[c]
	Infectivity on B-restricting spheroplasts	Infectivity on non-restricting spheroplasts		
None	1.6	9.1	0.09	0
B/1 extract				
50 μg protein	1.9	6.4	0.15	0.08
100 μg	2.8	4.6	0.31	0.24
CW-6 extract				
80 μg protein	1.2	5.5	0.11	(0.02)
160 μg	1.3	7.4	0.09	0.00
Pure enzyme				
0.75 μg protein	6.1	10.8	0.29	0.22

[a] Assays (70 μliters) were performed as previously described (6) with dialyzed ammonium sulfate fractions or purified enzyme (7) as indicated, except that 14 μM S-adenosylmethionine and 0.5 mM dithiothreitol (in place of mercaptoethanol) were present.

[b] OD_{260}, optical density at 260 nm.

[c] The ratio is normalized for the relative competence of the two spheroplast preparations (1.95) as determined with B-modified RF. As modification increases, the ratio increases proportionately from 0.09 to 1.0. The fraction of sites modified is estimated by dividing the increase of this ratio by 0.91.

TABLE 3. *Efficiency of plating of T7 lysates*

Phage \ Host	PFU/ml × 10^{-10}	
	B/1	CW-6
T7·B/1	2.06	2.02
T7·CW-6	2.75	2.98

possess sites that are acted upon by both the modification and restriction enzymes of *E. coli* B. Thus, the mechanism by which T7 escapes the action of these enzymes in vivo remains unknown; in fact, two unrelated mechanisms may be involved, since the restriction enzyme acts shortly after infection, whereas the modification enzyme acts during DNA replication (1).

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LITERATURE CITED

1. Arber, W. and S. Linn. 1969. DNA modification and restriction. Annu. Rev. Biochem. **38**:467-500.
2. Eskin, B., and S. Linn. 1972. The deoxyribonucleic acid modification and restriction enzymes of *Escherichia coli* B. II. Purification, subunit structure, and catalytic properties of the restriction endonuclease. J. Biol. Chem. **247**:6183-6191.
3. Eskridge, R. W., H. Weinfeld, and K. Paigen. 1967. Susceptibility of different coliphage genomes to host-controlled variation. J. Bacteriol. **93**:835-844.
4. Freifelder, D. 1970. Molecular weights of coliphages and coliphage DNA. IV. Molecular weights of DNA from bacteriophages T4, T5 and T7 and the general problem of determination of M. J. Mol. Biol. **54**:567-577.
5. Gefter, M., R. Hausmann, M. Gold, and J. Hurwitz. 1966. The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. X. Bacteriophage T3-induced S-adenosylmethionine cleavage. J. Biol. Chem. **241**:1955-2006.
6. Kühnlein, U., S. Linn, and W. Arber. 1969. Host specificity of DNA produced by *Escherichia coli*. XI. In vitro modification of phage fd replicative form. Proc. Nat. Acad. Sci. U.S.A. **63**:556-562.
7. Lautenberger, J. A., and S. Linn. 1972. The deoxyribonucleic acid modification and restriction enzymes of *Escherichia coli* B. I. Purification, subunit structure, and catalytic properties of the modification methylase. J. Biol. Chem. **247**:6176-6182.
8. Marvin, D. A., and B. Hohn. 1969. Filamentous bacterial viruses. Bacteriol. Rev. **33**:172-209.