JOURNAL OF VIROLOGY, May 1969, p. 541-542

Vol. 3, No. 5
Printed in U.S.A.

Mutant of φX174 Accessible to Host-controlled Modification

BARBARA SCHNEGG[1] AND PETER HANS HOFSCHNEIDER
Max-Planck-Institut fuer Biochemie, Munich, Germany

Received for publication 27 January 1969

A proflavine-induced φX174 mutant was isolated, φX174 sB1, which is accessible to restriction and modification by B-host specificity, but not by K-host specificity.

Host-controlled modification has been shown to occur with several bacteriophage strains carrying their genetic information on a single-stranded deoxyribonucleic acid (DNA) molecule (1). All these bacteriophage strains (fd, f1, M13, F12) are related inasmuch as their particles are rod-shaped, contain single-stranded DNA molecules of comparable size, and infect only male bacteria. Other single-stranded DNA phages unrelated to this group, such as φX174 and S13, are unable to infect cells of *Escherichia coli* K-12 and B, so that their sensitivity to K- and B- host specificity cannot be investigated directly. However, using spheroplasts of *E. coli* and infecting them with φX174 DNA molecules, Benzinger (3) showed that φX174 DNA is not restricted by B- or P1-host specificity. Lack of restriction of φX174 and the related phage S13 by P1-host specificity has also been shown by Eskridge, Weinfeld, and Paigen (4) by using the host pair *E. coli* C and C (P1).

In the experiments presented here, hybrids were selected from crosses with *E. coli* C and B (r_B^+ m_B^+), and C and K-12 (r_K^+ m_K^+), sensitive to infection by φX174 and carrying the genes responsible for B- and K-host specificity, respectively (5). With these hybrids, *E. coli* BC and KC, respectively, it is shown that wild-type φX174 is not susceptible to modification and restriction controlled by the genes responsible for λ DNA modification and restriction. The findings concerning B-host specificity thus confirm the results of Benzinger (3). However, a φX174 mutant was isolated which is accessible to restriction and modification in the hybrid *E. coli* BC.

Selection of E. coli KC and BC hybrids. Crosses were performed with (i) cells of Hfr K-12 951 (*thy*$^-$ *leu*$^-$) and (ii) cells of F$^+$ B 2027 (*met*$^-$ *leu*$^-$) as donor and cells of strain C_3 (*thr*$^-$) as recipient (Table 1). The *thr*$^+$ recombinants were selected on glucose-minimal agar plates. The recombinant cells were purified from donor and recipient cells by several consecutive steps of growth on minimal agar. The host specificity character of the *thr*$^+$ recombinant cells was determined by their ability to restrict and modify phage λ. Two types of hybrids were selected, KC r_K^+ m_K^+ and BC r_B^+ m_B^+, both sensitive to infection by φX174. These hybrids were investigated with respect to their ability to restrict and modify φX174. Wild-type φX174 was not restricted after infection of cells with K- and B-host specificity. The same result was found for the related phage S13 (Table 2).

Selection and characterization of a φX174 mutant sensitive to B-host specificity. Cells of *E. coli* C were infected with φX174 (10 phage particles/cell) and, after an adsorption time of 7 min, washed free from unadsorbed phage by two consecutive centrifugations in the cold. The cell-phage complexes were resuspended in tryptone broth and incubated with the addition of proflavine (10 μg/ml) for 2 hr at 37 C in daylight (G. Hobom, Ph.D. Dissertation, Munich, 1965). Progeny phage particles were plated on *E. coli* C to yield 15 to 30 plaques per plate. Phage particles (10^2 to 10^3) were then transferred from each plaque to cell lawns of strains KC and BC by replica plating with the aid of a stamp supplied with iron nails. Among 2,000 plaques investigated, one mutant was found sensitive to B-specific restriction. In detail, this mutant, shows the following characteristics. (i) The plaque morphology is identical to that of wild-type φX174. (ii) Like wild-type φX174, the mutant is able to infect cells of *E. coli* C, but not those of *E. coli* K-12 or B. (iii) It is inactivated by φX174 antiserum to the same degree as wild-type phages. (iv) The mutant grown on *E. coli* C or KC is accessible to restriction and modification in cells with B-host specificity (Table 2). The probability

[1] Present address: Department of Medicine (Infectious Diseases), Stanford University School of Medicine, Palo Alto, Calif. 94304.

TABLE 1. *Bacterial strains*

E. coli strain	Characteristics	Origin
C(P1)	P1 lysogen	Obtained after infection of *E. coli* C with P1 phage particles
C_3	r_0m_0 *thr*$^-$	HNO_2-induced mutant of *E. coli* C
K-12 951	Hfr $r_K^+m_K^+$ *thy*$^-$ *leu*$^-$	W. Arber
B 2027	F$^+$ $r_B^+m_B^+$ *met*$^-$ *leu*$^-$	W. Arber
KC	K ($r_K^+m_K^+$ *thr*$_K^+$)-C-hybrid	A *thr*$^+_K r^+_K m^+_K$-recombinant from cross K-12 951 × C_3
BC	B ($r_B^+m_B^+$ *thr*$_B^+$)-C-hybrid	*thr*$^+_B m^+_B$-recombinant from cross B 2027 × C_3

TABLE 2. *Plating efficiency of λ, φX174 wild-type and φX174 mutant sB1 on E. coli C, KC, BC, and C(P1)*

Phage type	Host specificity[a]			
	Cr_0m_0	$KCr_K^+m_K^+$	$BCr_B^+m_B^+$	C(P1)
λ.C	1	10^{-4}	10^{-4}	2×10^{-5}
λ.KC	1	1	10^{-4}	NT[b]
λ.BC	1	5×10^{-4}	1	NT
φX174.C	1	1	1	1
φX174.KC	1	1	1	NT
φX174.BC	1	1	1	NT
S13.C	1	1	1	1
φX174 sB1.C	1	1	5×10^{-3}	1
φX174 sB1.KC	1	1	5×10^{-3}	NT
φX174 sB1.BC	1	1	1	NT

[a] The efficiency of plating was determined by mixing 2.5 ml of peptone soft agar with 0.1 ml of an appropriately diluted phage suspension and 1 drop of indicator bacteria grown to the stationary phase in tryptone broth.

[b] Not tested.

of its growing on BC r_B^+ m_B^+ cells is 5×10^{-3}. Mutant genomes having escaped restriction in cells of strain BC give rise to phage particles able to grow on cells with B-host specificity with an efficiency of 1. Like the wild-type phage, the mutant is restricted neither in cells with K-host specificity nor by P1-host specificity. Thus, it may be concluded that the mutation introduced in the wild-type has originated at least one B specificity site. After the proposals of W. Arber and S. Linn (Annu. Rev. Biochem., *in press*), it should therefore be termed φX174sB1. That B specificity sites can be affected by single mutational events has been already shown by Arber and Kuehnlein (2). By one or two mutation steps, these authors could isolate mutants of wild-type phages fd, f1, and M13 no longer accessible to B-specific restriction and modification.

Because of the comparatively high spheroplast infectivity of phage φX174 DNA, the described mutant should provide a suitable system for studying restriction and modification of single-stranded DNA molecules in vitro. Furthermore, by using the mutant φX174sB1, it has been possible to demonstrate a transfer of parental φX DNA molecules to progeny phage particles at low multiplicities of infection. After infection of *E. coli* C with B-modified and density-labeled particles of φX174sB1, there appear phage particles among the progeny phages carrying the B-host specificity of the infecting parents and which are banding in CsCl density gradients at a position of intermediate density. (B. Schnegg and P. H. Hofschneider, *unpublished data*).

We thank Werner Arber for kindly supplying strains 951 and 2027 and Gisela Nass for phage P1. We thank W. Arber also for his criticism during preparation of the manuscript. We are indebted to Adolf Butenandt for his continuous interest.

This investigation was supported by the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

1. Arber, W. 1966. Host specificity of DNA produced by Escherichia coli. 9. Host-controlled modification of bacteriophage fd. J. Mol. Biol. 20:483–496.
2. Arber, W., and U. Kuehnlein. 1967. Mutationeller Verlust B-spezifischer Restriktion des Bakteriophages fd. Pathol. Microbiol. 30:946–952.
3. Benzinger, R. 1968. Restriction of infectious bacteriophage fd DNA's and an assay for in vitro host-controlled restriction and modification. Proc. Nat. Acad. Sci. U.S.A. 59:1294–1299.
4. Eskridge, R. W., H. Weinfeld, and K. Paigen. 1967. Susceptibility of different coliphage genomes to host-controlled variation. J. Bacteriol. 93:835–844.
5. Wood, W. B. 1966. Host specificity of DNA produced by Escherichia coli: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. 16:118–133.