RESTRICTION OF BACTERIOPHAGE ΦX174 BY F FACTOR

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SUMMARY

Bacteriophage ΦX174 is restricted in F^+ cells of Escherichia coli C. Restriction is causally linked to the acquisition of the episome and is lost on its removal. Phage produced in the restrictive host is not modified. Restriction occurs at some stage beyond adsorption and eclipse but does not appear to be due to a defect in phage release.

Plasmid-induced restriction of phage replication has been reported for F (sex), Col (colicine) and R (resistance) factors (1). Restriction by the F factor has been recorded for coliphages T3 (2), temperate phage tau (3) and phage ΦII (4). The restriction of phage ΦX174 by F factor is reported in the present paper.

MATERIALS AND METHODS

Bacterial strains and phage..Strains of Escherichia coli C designated CS and CT (both F^-) were obtained from Drs. R. L. Sinsheimer and I. Tessman respectively. The F^+ derivatives, CSF^+ and CTF^+, were made by transferring the episome from a male strain of K12. Dr. Sinsheimer supplied phage ΦX174.

Media and methods..Bacteria to be used as indicator strains were grown in LB medium (1% Tryptone [Difco], 1% NaCl, 0.5% yeast extract [Difco] and 0.1% glucose) to ca 10^9/ml after which $MgSO_4$ was added to a final concentration of 0.01 M. Bacteria for all other purposes were grown in Tryptone broth (1% Tryptone [Difco], 0.5% NaCl). Tryptone broth was also used as diluent.

Phage was assayed in an agar overlay system consisting of 25 ml Tryptone agar (1.5% agar) and 3 ml soft agar overlay (0.7% agar) with 3% glucose added

to the latter. The same plating system was used for bacterial counts except that glucose was omitted from the overlay. Phage adsorption was carried out in 0.01 M $MgSO_4$. A sample was then diluted into chloroform-saturated Tryptone broth and assayed for free, unadsorbed phage. Adsorbed phage was calculated as input minus free phage. In some experiments a lysozyme-EDTA freeze-thaw method (5) was used to determine free phage or to release intracellular phage. All procedures were carried out at 37° C except chloroform treatment and dilutions which were done at room temperature.

RESULTS AND DISCUSSION

Efficiency of plating (e.o.p.)..The relative plating efficiency of ΦX174 on two F^+ and F^- strains of E. coli C is given in Table I. In both cases the e.o.p. was significantly lower on the F^+ strain i.e. restriction was observed. Similar results were obtained when incubation was at 30° C. The possibility that either the phage stock or the F^+ strains were heterogenous mixtures was checked. Ten plaques were picked from plates on which CTF^+ was the indicator and the contents of each plaque were retitered on both CTF^+ and CT. The e.o.p. (CTF^+/CT) for the ten plaque isolates had a mean of 0.37 (range .33-.40) compared to the stock ΦX174 value of 0.33. The e.o.p. of

TABLE I. Efficiency of plating of ΦX174 on E. coli C.

Exp. no.	Efficiency of plating	
	CSF^+/CS	CTF^+/CT
1	.27	.22
2	.25	.15
3	.34	.17

The bacterial strains were grown as indicators and a suspension of phage was assayed on each.

ΦX174 on ten single clone CTF^+ isolates was also tested and the mean was 0.38 (range .34-.48) compared to 0.33 on stock CTF^+. Similar results were obtained with ten plaque and colonial isolates from CSF^+. Thus, both phage and bacterial populations were genetically homogenous with respect to restriction. It should be noted that though ΦX174 is restricted in the F^+ strain it is not modified by passage through it.

Relation of F episome to restriction..The causal relation of restriction to the F episome was tested and confirmed in two ways. Two fresh isolations of F^+ strains of CS and CT were made. The nine CSF^+ and twelve CTF^+ clones tested gave an e.o.p. with ΦX174 of less than 0.5 with individual values ranging from 0.26 - 0.5. Since there was considerable variation in the e.o.p. of a single strain from day to day, no significance is attached to the differences between the individual clones at this time. In the second type of experiment the method of Hirota (6) was used to isolate F^- revertants. Revertants were identified as clones which had lost sensitivity to male specific RNA phages, MS2 and f2. Revertants from strain CTF^+ gave the same e.o.p. as the original CT strain. Efforts to isolate revertants of CSF^+ have failed so far.

Nature of restriction..Experiments were performed in which ΦX174 was preadsorbed to both F^+ and F^- strains of CS and CT after which the infected cells were plated on a common indicator, CS. Results are given in Table II. Adsorption to all strains was over 99% complete under the conditions described and eclipse occurred as measured by the chloroform technique and subsequently by the lysozyme-EDTA method. Since restriction occurred under these conditions it is evident that restriction is not due to interference with these early steps in infection, though a defect in DNA penetration is not excluded. Restriction was observed in both actively growing and stationary phase cells with some indication that stationary phase CTF^+ cells are more restrictive than log phase cells.

The possibility that mating on the plates was in some way related to restriction was excluded in control experiments in which it was shown that in-

TABLE II. Efficiency of plating of ΦX174 under various conditions.

Cell state	Efficiency of plating			
	CSF^+/CS		CTF^+/CT	
	1	2	1	2
Log	.63	.43	.47	.47
Log-starved	.50	.50	.43	.39
Stationary	.42	.35	.21	.18
Stationary-starved	.50	.40	.29	.31

Bacterial strains were grown in Tryptone broth with aeration to log phase (ca. 4×10^8/ml) and to stationary phase (ca. 1×10^9/ml) after which the cultures were washed once and resuspended in 0.01 M $MgSO_4$ to ca. 10^8/ml. One sample was infected immediately and a second was starved for 60 minutes at 37° C with aeration. Phage was added to the cells (phage/bacteria : 1/20), and after ten minutes adsorption at 37° C free phage and total infective centers was determined. *E. coli* C strain CS was used as the indicator strain for all assays.

fected F^+ and F^- cells gave the same relative e.o.p. on F^+ and F^- indicators. The possibility that restriction of ΦX174 was due to a defect in lysis rather than in replication was also considered. If this were the case the yield of ΦX174 from F^+ infected cells should increase dramatically following artificial lysis. However, phage yields from both F^+ and F^- cultures showed similar increases after artificial lysis indicating that lysis inhibition does not explain restriction. In addition, as shown below, some infected F^+ cells may recover their colony forming ability, an unlikely result if restriction were due to lysis inhibition.

Finally the effect of multiplicity of infection on restriction and the fate of the restricting cells was determined. The results in Table III show that a relatively constant proportion of F^+ cells can produce phage over a

TABLE III. Effect of multiplicity of infection on restriction and restricting host.

Multiplicity of infection	Fraction of infected cells producing	
	Plaques	Colonies
0.60	.32	.79
0.74	.43	.51
0.96	.26	.83
1.4	.31	.42
1.7	.31	.49
2.6	.35	.30
2.7	.30	.36
3.1	.37	.35
4.0	.27	.43
5.5	.35	.24
6.4	.35	.10
7.8	.35	.15
8.1	.30	.17

Cells were grown to stationary phase, washed, resuspended and starved as described in Table II. Phage was added at various multiplicities and after 10 minutes adsorption at 37° C free phage was assayed and the mixture was diluted into 0.01 M $MgSO_4$ containing ΦX174 antibody. After 6 minutes, during which over 99% of the free phage was neutralized, the mixture was diluted and assayed for total infective center, free phage and colony forming units. Strain CS was used for all phage assays.

range of multiplicities of 0.6 - 8. This indicates that there are two physiologically distinct classes of cells in the F^+ culture; one can support phage replication and the other cannot. The data on colony forming units show that at lower multiplicities of infection a high proportion of infected cells apparently recover from infection, but as the multiplicity increases such infections are lethal. Further study is needed to determine whether recovery from infection is correlated with the presence of the episome.

In summary, the data show that restriction of ΦX174 in F^+ strains occurs at some stage of infection following eclipse. This could include the penetration of DNA as well as some stage in phage replication or maturation. The degree of restriction observed is small compared with other systems, but Schell

et al (2) also noted that the F^+ strain of E. coli C caused less restriction of T3 phage (e.o.p. 10^{-1}) than K12F^+ (e.o.p. 10^{-5}). We have observed that an R factor which suppresses fertility (fi^+) also restricts ΦX174 when introduced into E. coli C. The effect of other R factors and plasmids on restriction remains to be studied. We have also observed that lambda phage plaquing efficiency is unaffected by the F episome, therefore restriction appears to have some specificity.

Restriction or modification of ΦX174 was not observed until recently. Eskridge, Weinfeld, and Paigen (7) failed to observe restriction of ΦX174 and two similar phages, S13 and F1, by P1 host specificity, and Benzinger (8), using phage DNA and appropriate host spheroplasts, showed that neither P1 nor B host specificity was restrictive for ΦX174. However, Schnegg and Hofschneider (9) recently described a mutant of ΦX174 which was restricted and modified in cells with B host specificity, and Zamenhof (10) reported that ΦX174 was restricted but not modified by a strain of E. coli C which had acquired a mutator gene. The mechanism of restriction or modification of ΦX174 is not known for any of the cases reported.

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