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A Coat Protein from Bacteriophage fd

III. Specificity of Protein–DNA Association *in vivo*

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Phage fd and phage ϕX174 multiply without mutual interference in a common host cell. About 3% of the phage progeny contain ϕX174 DNA with an fd protein coat and about 0·03% of the progeny contain fd DNA with a ϕX174 protein coat. There is thus only a limited specificity of fd protein–DNA association *in vivo*.

1. Introduction

The coating of DNA with fd protein *in vitro* has been found to be species specific in the sense that infectious virus was generated only from fd DNA and not from ϕX174 DNA (Knippers & Hoffmann-Berling, 1966*b*). These types of DNA closely resemble each other in molecular size, secondary structure, ring topology and the proportions of the constituent nucleic acid bases (Sinsheimer, 1959; Fiers & Sinsheimer, 1962; Hoffmann-Berling, Marvin & Dürwald, 1963; Marvin & Hoffmann-Berling, 1963; Marvin & Schaller, 1966). The specificity did not originate at the level of the DNA–protein interaction, but from a directing influence of fd-coat structures on ordered fd protein–DNA assembly. Residual fd-coat structures persisted in the phenol-extracted fd DNA but were, of course, lacking in the DNA extracted from ϕX174. In order to characterize further the specificity and to elucidate whether in addition species specific DNA–protein association exists, cells infected by both fd and ϕX174 were studied and the frequency of erroneous incorporation of ϕX174 DNA in fd-protein coats determined.

Incorporation of viral nucleic acid into heterologous capsids as a result of mixed infection with two viruses has been repeatedly observed. This has been termed "genomic masking" (Yamamoto & Anderson, 1961), in order to distinguish the phenomenon from phenotypic mixing, i.e. the coexistence of protein elements from different viruses in a common viral shell. Genomic masking involving completely unrelated viruses has been reported in one case. Easton & Hiatt (1965) showed the masking of SV 40 genomes by adenovirus 4 protein coats. Coating of turnip yellow mosaic virus RNA and phage R17 RNA with tobacco mosaic virus protein has been achieved *in vitro*, but the products were non-infectious when tested in hosts receptive to either tobacco mosaic virus or to one of the viruses from which the RNA had been derived (Matthews & Hardie, 1966).

2. Materials and Methods

(a) *Media*

Modified Tryptone medium has been described (Knippers & Hoffmann-Berling, 1966*a*). Dilution buffer is 0·1 M-NaCl, 0·005 M-$MgCl_2$ and 0·01 M-Tris–HCl (pH 7·5).

(b) *Phages and bacteria*

Purified preparations of fd (Hoffmann-Berling *et al.*, 1963) and ϕX174 (Rueckert, Zillig & Huber, 1962) were used.

Escherichia coli host strains were: (1) K12 W945 (F^+), sensitive to fd; (2) C, sensitive to ϕX174; (3) C(F^+), sensitive to both ϕX174 and fd. Phage ϕX174 plates on strains C and C(F^+) with the same efficiency; fd plates on C(F^+) with an efficiency of 0·8 compared to K12 = 1·0, whether the phage had been grown on K12 or on C(F^+).

(c) *Mixed infection with fd and ϕX174*

There is no evidence of competition between fd and ϕX for common receptors on *E. coli* C(F^+) (Knippers & Hoffmann-Berling, 1966*a*). Mixed infection with both types of phages was carried out in two ways, both intended to compensate for the slower adsorption of fd (Hoffmann-Berling, Dürwald & Beulke, 1963) relative to ϕX (Sinsheimer, Starman, Nagler & Guthrie, 1962).

(i) *Simultaneous infection*

E. coli C(F^+) was grown in modified Tryptone medium at 37°C to 2×10^8 cells/ml. Then 60 to 100 PFU† fd and 10 to 20 PFU ϕX174 were added per cell and the culture was further aerated for 8 min. The cells were spun down, washed 3 times with cold dilution buffer to remove non-adsorbed phage and resuspended in the original volume of warm medium.

(ii) *Super-infection of fd-pre-infected cells*

E. coli C(F^+), grown to 1×10^8 cells/ml., was infected with 3 fd/cell. After 20 min, the cells were diluted 1 : 20 in warmed medium and grown to 2 to 3×10^8 cells/ml. Controls had shown that cultures at this time had become completely infected. For super-infection, 5 ϕX174/cell were added.

Samples from the cultures were diluted through a mixture of fd antiserum and ϕX antiserum and plated, using *E. coli* K12 and *E. coli* C respectively for parallel determinations of phage. Plaque counts were compared to bacterial counts obtained in a Petroff–Hausser chamber. It was found that both infection techniques led to a mixed infection of 90 to 95% of the cells.

(d) *Preparation of progeny phage*

In some of the experiments, doubly infected cells were allowed to undergo ϕX-induced bacterial lysis. Higher phage yields were obtained by sedimenting the cells 16 min after addition of ϕX174 and resuspending the pellet from a 20-ml. culture in 3 ml. 0·01 M-Tris–HCl (pH 8·1). 0·1 ml. of lysozyme (Worthington A grade, 2 mg/ml.) and 0·1 ml. of 0·1 M-Na_2H_2EDTA were then added. After 10 min at 37°C, the mixture was frozen and thawed 3 times. Cellular debris was removed by low-speed centrifugation. More than 80% of the phage was recovered in the supernatant fraction.

(e) *Equilibrium density centrifugation*

Density distribution of phage progeny was carried out by diluting phage suspensions in buffer to about 10^6 PFU/ml.; 4·5-ml. samples were brought to a density of 1·35 g/ml. by adding caesium chloride (Merck, Darmstadt) and centrifuged in an SW39 rotor of the preparative Spinco ultracentrifuge for 24 hr at 38,000 rev./min and +4°C. Three-drop fractions collected from the gradient were diluted in 1 ml. of sterile buffer.

† Abbreviation used: PFU, plaque-forming units.

(f) *Assay for intracellular viral DNA*

A 20-ml. culture of *E. coli* C(F^+) containing $1{\cdot}5 \times 10^8$ cells/ml. was infected with ϕX174 and fd. After being aerated for 16 min, the cells were spun down, washed once with cold dilution buffer, suspended in 2·5 ml. 0·01 M-Tris–HCl and lysed as described. The solution was aspirated several times through a pipette to reduce the viscosity. A 3-ml. sample, brought to a density of 1·705 g/ml. by adding 3·78 g caesium chloride, was then centrifuged for 48 hr at 18°C and 38,000 rev./min in the SW50 rotor. Four-drop fractions were collected from the gradient and added to 0·5 ml. of 0·01 M-Tris–HCl (pH 7·5). 0·1-ml. samples were titrated, after appropriate dilution (1:30), in the spheroplast system described by Guthrie & Sinsheimer (1963). The appropriate bacterial indicator was used to count either fd DNA- or ϕX174 DNA-induced infections. Results were normalized for single-strand equivalents of DNA by comparison with a standard DNA preparation obtained from phage, assuming equal specific infectivities for DNA single strands isolated from cells and from virus. The specific infectivity in the Guthrie–Sinsheimer system is 10^{-3} infective centre/phage-equivalent DNA for ϕX174 DNA, and 10^{-5} for fd DNA.

(g) *Other techniques*

Single-burst experiments were conducted as described by Adams (1959). Phage was plated according to the same author. Antiserum for ϕX174 was a generous gift of Dr W. Zillig. Antiserum for fd was prepared as described previously (Knippers & Hoffmann-Berling, 1966*a*).

3. Results

(a) *Phage growth in mixedly infected cells*

E. coli C(F^+) cultures, pre-infected with fd and super-infected with ϕX174, continued to increase in tubidity and to release fd at a normal rate of about 100 phages/cell per cell generation (Fig. 1). Independent experiments had shown the pool of intracellular

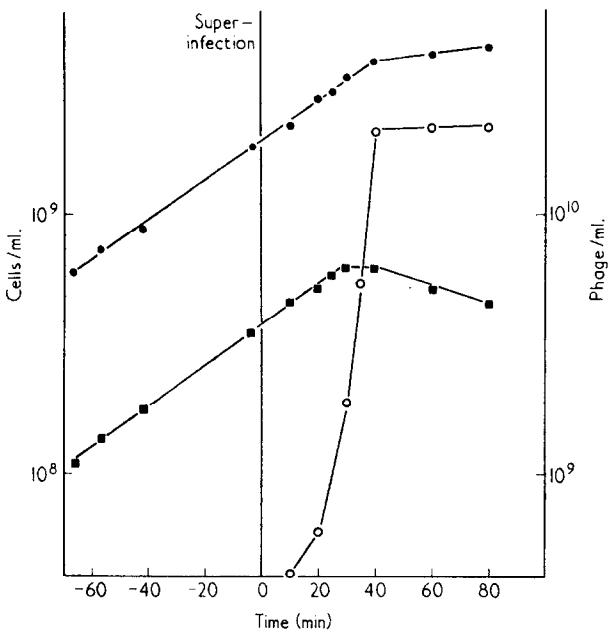

FIG. 1. Growth curve of a mixedly infected *E. coli* C(F^+) culture.

An *E. coli* C(F^+) culture in modified Tryptone medium at 2×10^8 cells/ml. was infected with phage fd with a multiplicity of 2. 20 min later, a 1-ml. sample was diluted into 19 ml. of warmed medium. At a cell concentration of $3{\cdot}7 \times 10^8$/ml. (time: 0 min), phage ϕX174 was added at a multiplicity of 3·6. Cell concentrations (—■—■—) were determined from turbidity measurements at 460 mμ. Phage fd (—●—●—) was plated in appropriate dilution on *E. coli* K12 and ϕX174 (—○—○—) on *E. coli* C.

fd to be very small (less than 5 phages/cell). Therefore the majority of the fd particles released from a ϕX174 super-infected culture should be formed after super-infection. Super-infected cells started to release ϕX174 after the normal latency period of about 20 minutes (Rueckert & Zillig, 1962). Visible lysis occurred at about 30 minutes. Yields of ϕX174 under conditions of mixed infection were between 60 and 120 phages per cell. Conclusive evidence for a joint multiplication of fd and ϕX174 in a common host cell with no mutual interference came from an evaluation of single bursts.

In these experiments, *E. coli* C(F^+) cells were diluted, seven minutes after simultaneous infection with fd and ϕX174, first into an antiserum mixture to inactivate extracellular phages. Then the cells were diluted to give 0·2 cell/ml. In each experiment 60 ml. of the final dilution were distributed in 60 tubes containing medium. One hour later, samples of each tube were plated for progeny phage on *E. coli* C and on *E. coli* K12, respectively. Out of 100 infected cells, about 96 cells had a mixed infection. These cells produced an average of 185 ϕX phages and 324 fd phages/cell.

No systematic search for the occurrence of fd–ϕX genetic crosses was undertaken. A ϕX174 plaque type–fd host range recombination was looked for but could not be detected.

(b) *Determination of free DNA molecules in mixedly infected cells*

The frequency of genomic masking should be influenced by several factors, including the relative size of the precursor DNA pools in their common host. In order to estimate the size of these pools, cells were opened 16 minutes after mixed infection and the crude extract was banded at an initial density of 1·705 g/ml. (Fig. 2). Spheroplast infectivity of the fd type and the ϕX type each appeared in two bands. Corresponding bands centred around the same density, as would be expected for DNA species of very similar nucleotide composition and secondary structure. The heavier bands were observed at $\rho = 1{\cdot}725$ g/ml. (fractions 12 to 19) and from their density position were assumed to contain the single-stranded viral DNA species (ϕX single-strand infectivity was split into two minor bands reproducibly). Judging from spheroplast titration and accepting the assumptions mentioned under Materials and Methods, single-stranded DNA pools generated by fd and ϕX in a common host are about equal in size, comprising approximately 6 and 12 phage equivalents per cell, respectively. Pools of a similar size have been found in C(F^+) cells infected with either of these phages and, as far as ϕX is concerned, have been reported by Sinsheimer *et al.* (1962) and Denhardt & Sinsheimer (1965).

In the density-gradient represented in Fig. 2, a second band of infective fd DNA appeared (fractions 24 to 34), which centred around the density of ϕX-replicative form DNA ($\rho = 1{\cdot}705$ g/ml.); this may be considered as a double-stranded intermediate of fd DNA replication. Evidence for a double-stranded replicative form of fd DNA has also been obtained by Bonhoeffer & Marvin (personal communication) and for the DNA of the closely related phage M13 by Hofschneider & coworkers (Hofschneider, personal communication).

(c) *Genomic masking*

Artificial lysates obtained from fd pre-infected and ϕX super-infected bacterial cultures were subjected to density equilibrium centrifugation in caesium chloride at an initial density of 1·35 g/ml. Phage ϕX174 ($\rho = 1{\cdot}40$ g/ml.) banded near the bottom of the tube and fd ($\rho = 1{\cdot}29$ g/ml.) near the meniscus. Individual fractions

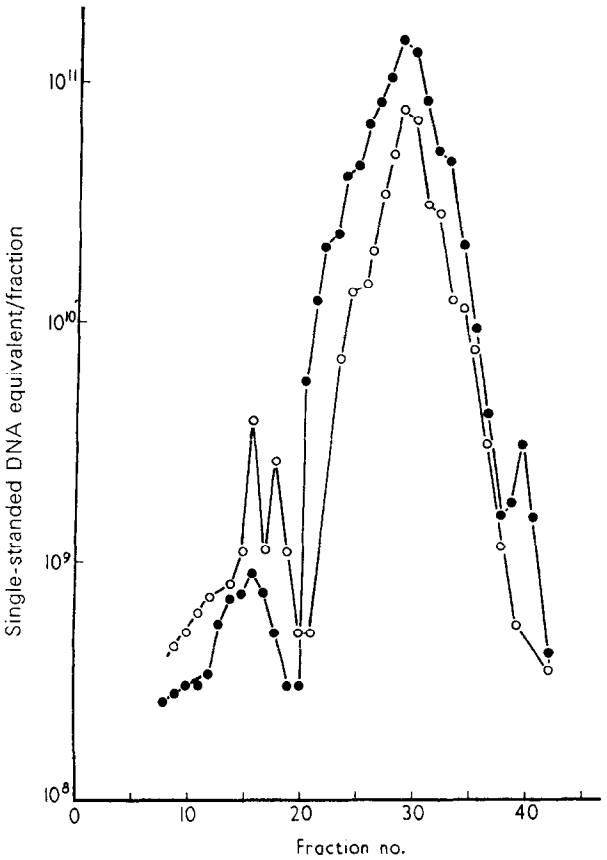

FIG. 2. Density distribution of intracellular phage DNA of mixedly infected *E. coli* C(F^+) cells.

Infective centres were evaluated on K12 for fd DNA (—●—●—) and on *E. coli* C for ϕX DNA (—○—○—). Plating results were compared to the plaque yield of DNA solutions of known concentration and expressed in single-stranded DNA equivalents per fraction. Initial density: 1·705 g/ml.

from the gradient were each plated, after appropriate dilution, on *E. coli* C, *E. coli* C(F^+) and *E. coli* K12, respectively. Infective particles appeared in two bands (Fig. 3). An fd satellite band near the meniscus and the asymmetry of the two light bands may result from sampling errors.

More than 99·9% of the particles in the heavier band were normal ϕX, judged from their buoyant density, host specificity, plaque morphology, sensitivity to ϕX antiserum and resistance to fd antiserum. The particles of the lighter band, according to their plague type, were predominantly fd. When plated on *E. coli* C(F^+) instead of *E. coli* K12, however, a smaller number of typical ϕX plaques appeared. Such plagues could not be detected by plating directly on *E. coli* C, but appeared after pre-adsorption to K12 cells and plating on *E. coli* C as an indicator. The corresponding infectious particles were inactivated by fd antiserum but not by ϕX antiserum, and showed the high sensitivity to ultrasonic inactivation which distinguishes fd from resistant ϕX174 (Fig. 4).

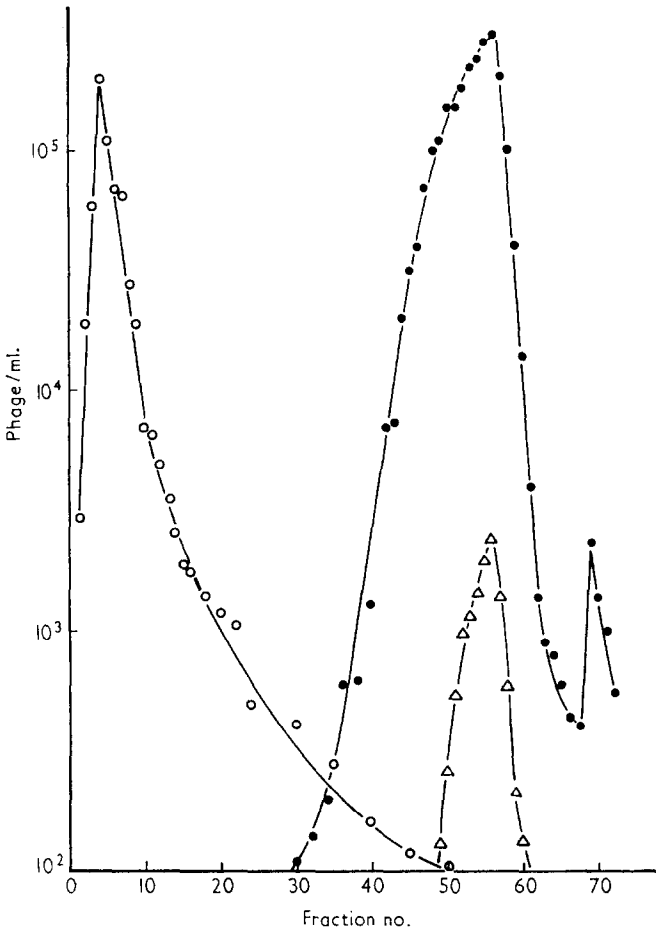

FIG. 3. Density distribution of an artificial lysate of mixedly infected cells.

Lysate was diluted 1:1000 before centrifugation. Initial density was 1·35 g/ml. Phage fd as plated on K12 (—●—●—); ϕX174 as plated on C (—○—○—); particles composed of ϕX DNA and fd-coat protein (—△—△—).

Phage progeny obtained by stabbing ϕX plaques formed on a C(F^+) lawn were typical ϕX in all respects tested. The original particles thus had phenotypic host specificity of fd but gave rise to progeny with the host specificity and the plaque morphology of ϕX. It appeared that the particles contained ϕX genomes wrapped in fd protein.

The number of ϕX genomes masked by fd-coat protein compared to those wrapped in the homologous protein differed somewhat from experiment to experiment. Usually 2 to 4% of all ϕX genomes had the non-homologous protein.

Particles which had, *vice versa*, an fd DNA core and a ϕX protein coat were more difficult to find, since ϕX-induced lysis of the C(F^+) lawn obscured fd plaques. But after pre-adsorption of the phages present in the heavier band of Fig. 3 on *E. coli* C and plating on K12 indicator cells, a certain number of fd plaques were always found. No such fd plaques were found if the samples were treated with ϕX antiserum before pre-adsorption. If the fd plaques found after pre-adsorption on *E. coli* C

reflected the number of fd DNA particles masked by ϕX coat protein, then 0·02 to 0·05% of the fd DNA molecules present were usually wrapped in the non-homologous protein.

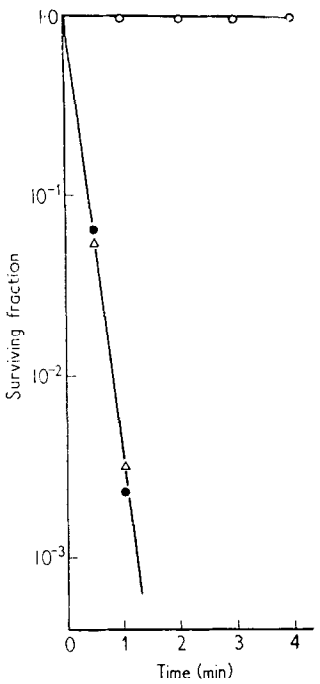

FIG. 4. Inactivation of ϕX DNA–fd-coat particles by ultrasonic treatment (—△—△—). Control experiments with phage ϕX174 (—○—○—) and phage fd (—●—●—).

4. Discussion

The results demonstrate that single-stranded ϕX174 DNA can be wrapped in non-homologous coat protein. If the co-aggregation of DNA and protein in the fd- and ϕX-producing cell was random, then 50% of the phage progeny should show genomic masking. The smaller portion actually found may reflect some inherent specificity in DNA–protein co-aggregation in the living cell. Since nothing is known about the intracellular locus of phage multiplication, it is also possible that fd progeny and ϕX174 progeny are formed in different compartments of the cell. In this case an exchange of viral structural components would be reduced.

The difference in the yield of genomic masking of fd DNA and of ϕX174 DNA is also unexpected. One only can tentatively assume that a larger amount of fd protein is present in the cell, or that the assembly of an fd-like coat proceeds at a higher rate than that of a ϕX174-like protein coat. Other explanations—for example, that fd DNA is slightly larger than ϕX DNA and cannot be easily encased in a ϕX coat—are also feasible.

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