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Bacteriophage f1 Infection: Fate of the Parental Major Coat Protein

HENRY SMILOWITZ

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111

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The major coat protein of infecting f1 phage is incorporated into the inner membrane of the host cell, even in the absence of phage f1DNA penetration and replication. The major coat protein monomers are reutilized in the assembly of new phage. They are not conserved as a single unit but behave as independent units which are slowly incorporated into newly manufactured phage.

The fate of the protein component of the filamentous DNA bacteriophage f1, fd, or M13 during the infection process has been the subject of some controversy. Trenkner, Bonhoeffer, and Gierer (11) previously reported that the whole virus invades the host cell upon infection. However, the amount of protein label associated with the cell was very low and their result was difficult to interpret. They also performed an experiment in which phage labeled with ^{14}C-amino acid and 5-bromodeoxyuridine were used to infect cells; a considerable portion of the radioactively labeled protein which was initially associated with the heavy phage was found in a light phage band, implying that the radioactive coat protein associated with the cell upon infection was reutilized in some manner by progeny phage.

Four years later Henry and Brinton (2) were unable to remove reproducibly and quantitatively the cell-associated protein derived from M13 phage. They therefore concluded that some of the parental coat subunits might enter the cell, be broken down to amino acids, and be partially reutilized by progeny phage.

In this paper we show that about 50 to 60% of the major coat protein of infecting phage becomes specifically and functionally associated with the host cell inner membrane upon phage infection. These results are discussed in light of our previous findings that the newly made f1 major coat protein becomes specifically and functionally associated with the host cell inner membrane (9).

MATERIALS AND METHODS

Bacteria. *Escherichia coli* K37 and *E. coli* R141 suI B were obtained from N. D. Zinder and Peter Model of the Rockefeller University. *E. coli* A600 [W4032 (P1d1) Sm^s lac$^-$ F+] and *E. coli* A602 [W4032 (P1d1) Sm^s Tol II F$^+$] were obtained from S. E. Luria of the Massachusetts Institute of Technology (MIT).

Bacteriophage. f1 am$^+$, SB=0 (SB$_1$° SB$_2$°) and SB=1 (SB$_1$°) were obtained from Peter Model and N. D. Zinder of the Rockefeller University.

Chemicals. [^{14}C]tyrosine (300 mCi/mmol), [^{14}C]lysine (200 mCi/mmol), [*methyl*-^{3}H]thymidine (6.7 Ci/mmol), and [^{3}H]leucine (5 Ci/mmol) were purchased from the New England Nuclear Corp. Deoxyadenosine was purchased from Schwartz Bioresearch, Inc., cesium chloride was purchased from the Harshaw Chemical Co., Solon, Ohio, and lactoperoxidase was purified from raw milk by the procedure of Morrison and Hultquist (4) and was the generous gift of Gary Wickus of MIT. The A_{412}/A_{280} ratio of the enzyme was 0.435.

Phage infection, growth, and purification. Exponentially growing cells were infected at a multiplicity of infection of 100 at 37 C; phage were grown and purified by the method of Rossomando and Zinder (6).

Manufacture of [^{3}H]thymidine- and [^{14}C]lysine-labeled phage. An infected cell culture was grown in M9 medium (7) supplemented with 0.4% glucose, 250 μg of deoxyadenosine per ml, and 19 amino acids (each at 20 μg/ml). Lysine was omitted. The culture was filtered on a membrane filter (0.45 μm pore size, Millipore Corp.), washed with M9 medium, and resuspended in M9 lysine-free medium containing 250 μg of deoxyadenosine per ml, 1 mCi of [^{3}H]thymidine, and 10 μCi of [^{14}C]lysine. Phage were purified after 1 h of incubation at 37 C. The phage made contained 2.4×10^6 counts/min of [^{3}H]thymidine and 2×10^5 counts/min of [^{14}C]lysine in 10^{12} phage.

Manufacture of [^{3}H]leucine, [^{3}H]lysine, and [^{14}C]lysine phage. An infected cell culture growing in M9 leucine-free medium or M9 lysine-free medium was filtered on a membrane filter (0.45μ) washed with M9 medium and resuspended in M9 leucine-free medium or M9 lysine-free medium containing 100 μCi of [^{3}H]leucine or 500 μCi of [^{3}H]lysine or 20 μCi of [^{14}C]lysine. Phage were purified after 1 h of incuba-

tion. The phage made were as follows: [^{3}H]leucine SB=0 (3.4×10^6 counts/min in 4.5×10^{12} phage), [^{3}H]leucine SB=1 (1.6×10^7 counts/min in 8×10^{12} phage), ^{3}H-lysine SB=0 (1.3×10^7 counts/min in 9×10^{11} phage), ^{3}H-lysine SB=1 (9.4×10^6 counts/min in 1.5×10^{12} phage), and [^{14}C]lysine SB=1 (2.3×10^6 counts/min in 7.7×10^{11} phage).

Association of parental phage major coat protein and DNA with the host cell. Exponentially growing cultures of *E. coli* were infected with labeled phage for the desired length of time. The cells were centrifuged at 10,000 × *g* for 10 min and blended in a Waring blender for 1 min at 0 C. This process was repeated twice. (Two cycles of washing and blending removed 25 to 40% of the label initially associated with the cell. Further washing and blending had little effect.) The twice blended and centrifuged cells were assayed for their content of radioactivity. At least two cycles of blending and washing are necessary to remove 90% of the adventitiously bound phage.

Preparation of heavy-iodinated phage. The reaction mixture contained in 1 ml of M9 medium 60 μg of lactoperoxidase, 2 to 20 μmol of potassium iodide, 0.1 to 10 mg of substrate protein, and 100 nmol of H_2O_2. These conditions were chosen after several preliminary experiments had been performed to determine optimal enzyme-catalyzed iodination.

Preparation of inner and outer membrane. Inner and outer membranes were prepared by the method of Smilowitz, Carson, and Robbins (9).

Cesium chloride density gradients. Each sample was prepared by layering 1.5 ml of sample (usually in M9 medium) on 4 ml of 40% (wt/wt) cesium chloride in cellulose nitrate tubes. The gradients were formed by spinning the mixture in an SW39 or SW50.1 rotor for 24 to 36 h at 34,000 rpm. The gradients were analyzed by puncturing the bottom of one tube and collecting three drops per scintillation vial. Each vial was counted in Triton-toluene scintillation fluid.

RESULTS

Association of parental phage coat protein with host cell inner membrane. *E. coli* A600 is sensitive to phage f1. The colicin-tolerant mutant *E. coli* A602 appears to be blocked in f1 DNA penetration (8). *E. coli* A600 and A602 were infected with [^{3}H]thymidine, [^{14}C]-lysine doubly labeled f1 phage in the presence or absence of KCN. (Recent reports indicate that F pili fall off after KCN treatment [5]; hence, KCN treatment reflects the nonspecific sticking of phage to cells). After two successive shearing and washing treatments (see Materials and Methods), the cells were assayed for radioactivity. Table 1 shows that both the [^{14}C]lysine label and the [^{3}H]thymidine label associate with the wild-type cells. The ratio of label found is almost identical to the ratio of label found in whole phage. KCN was seen to block the association of both [^{3}H]thymidine and [^{14}C]lysine with the infected cells by about 93 to 94%. [^{3}H]thymidine-labeled phage DNA does not associate with *E. coli* A602, but [^{14}C]lysine-labeled phage coat does. This association is largely blocked by KCN in the colicin-tolerant mutant. Hence the association of infecting phage coat with the colicin-tolerant cells is not dependent upon phage DNA penetration or replication. This is also supported by the fact that the association of labeled parental phage coat with the host cell is nearly normal when wild-type or mutant cells are infected in the presence of rifampin or chloramphenicol. This is true even after 8 min of preincubation with the drugs before phage addition. Rifampin has been shown to block M13 DNA replication (1); chloramphenicol blocks protein synthesis.

TABLE 1. *Association of infecting phage coat with host cell in the absence of DNA penetration*[a]

Culture	Radioactivity associated with cells (counts/min)	
	^{3}H	^{14}C
(A) A600	92,000	6,560
(B) A600 + KCN	5,600	480
(C) A602	6,600	4,840
(D) A602 + KCN	2,000	1,200

[a] An 80-ml amount of *E. coli* A600 and 80 ml of *E. coli* A602 were grown in LB broth. Each culture was split into two parts. One culture was treated with 0.02 M KCN for 5 min. Both cultures were injected with 2×10^{11} double-labeled phage (2.4×10^6 [^{3}H]thymidine counts/min; 2×10^5 [^{14}C]lysine counts/min in 10^{12} phage). Cultures were sheared in a Waring blender twice and centrifuged twice at 10,000 × *g* for 10 min. The twice blended and centrifuged cells were assayed for their content of radioactivity.

Membranes were prepared from *E. coli* A600 and *E. coli* A602 infected with [^{3}H]thymidine, [^{14}C]lysine phage by the procedures described in Materials and Methods. Cytoplasm and pellet (envelope fraction) were assayed for radioactivity. In each experiment 50 to 60% of the [^{14}C]lysine counts were recovered in the membrane fraction. Further, membranes prepared from *E. coli* A600 were centrifuged on sucrose density gradients and assayed for radioactivity. The results of sucrose density gradient fractionation of the membranes shown in Fig. 1A indicate that all of the lysine and thymidine label found in these particulate fractions appears in the inner membrane region of the gradient; an outer membrane band was visible on this gradient but it contained no radioactivity. To show that this label is not in intact phage (intact phage band on the heavy side of the inner membrane region on these gradients

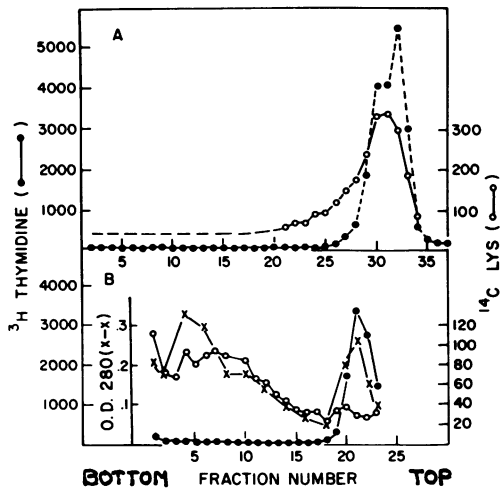

FIG. 1. *Association of infecting phage major coat protein with host cell inner membrane. A, Inner and outer membranes were prepared from the E. coli A600 culture described in Table 1. The method for separating inner and outer membranes is described by Smilowitz, Carson, and Robbins (9). The radioactivity is shown all along the gradient. B, The inner membrane region from part A was pooled, dialyzed against 5 mM EDTA and adjusted to 10 mM Mg^{2+} plus 10 μg of DNase per ml. After 30 min of incubation at 37 C, the membranes were rerun on a sucrose density gradient. Radioactivity was assayed along the gradient. [^{3}H]thymidine (●); [^{14}C]lysine (○); OD_{280} was followed along the gradient (×).*

[9]), the inner membrane fractions were pooled, treated with DNase and rerun on a sucrose density gradient. Figure 1B shows that the [^{14}C]lysine and [^{3}H]thymidine counts per minute are separated by this procedure. While the [^{14}C]lysine counts per minute do not appear in the normal inner membrane region of the gradient, they coincide with the bulk of the UV absorbing material (optical density at 280 nm) indicating that they are still associated with the inner membrane. In some way Mg^{2+} and DNase treatment causes the inner membrane to band anomalously.

Reutilization of parental phage coat by newly replicated phage. We have taken advantage of a suggestion made by Hugh Robertson of the Rockefeller University to use amino acid-labeled f1 phage restriction mutants to study the reutilization of parental phage coat by progeny phage. The protagonists in this experiment are the restriction mutants SB=0 and SB=1. The mutant SB=0 contains mutations in both restriction sites of this phage and therefore can plate with the same efficiency on *E. coli* B and *E. coli* K. In contrast, SB=1 contains a mutation in only one restriction site. SB=1 phage can only form about 2 to 3% of the number of plaques possible on *E. coli* K when plated on *E. coli* B. We reasoned that, if parental phage coat protein is reutilized by newly replicated progeny phage, then *E. coli* B cells infected with labeled SB=1 phage should yield progeny phage which contain a small amount of radioactivity; *E. coli* B cells infected with labeled SB=0 phage should yield many more progeny phage which contain much more radioactivity. Alternatively, if labeled parental phage merely stick to cells and then dissociate from the cells, the progeny produced from both infections will contain comparable amounts of radioactivity.

Therefore, we infected one broth-grown culture of *E. coli* with [^{3}H]leucine-labeled SB=0 and one culture with [^{3}H]leucine-labeled SB=1. After extensive washing and blending to remove adventitiously bound phage, the infected cells were allowed to grow for 3 h at 25 C, a temperature at which phage infection is severely limited while phage production is normal. Phage were recovered from the growth medium and assayed for their content of [^{3}H]leucine label as well as their ability to grow on *E. coli* B and *E. coli* K. The results are shown in Table 2. Of the 74,000 counts/min initially found associated with *E. coli* B cells after infection with [^{3}H]leucine-labeled SB=0 (about 1 phage per cell), 23% was recovered in progeny phage, 22% remained associated with the host cells as phage coat (determined by polyacrylamide gel electrophoresis), and 55% left the cell as phage coat (as determined by polyacrylamide gel electrophoresis) but was not recoverable in phage particles. All of the phage produced in this infection were recovered upon phage purification and they formed plaques equally well on *E. coli* K and *E. coli* B as did the parental SB=0 phage. Of the 156,000 counts derived from SB=1 phage coat which became associated with the *E. coli* B cells (about 1 phage per cell), only 2.5% of the counts were found in progeny phage after 3 h of growth at 25 C; 54% of the radioactivity remained associated with the host as phage coat (as determined by polyacrylamide gel electrophoresis), whereas 43% left the cell as phage coat (as determined by polyacrylamide gel electrophoresis) but was not recoverable in phage particles. Here too, all of the phage produced formed plaques equally well on *E. coli* K and *E. coli* B, unlike the parental SB=1 which was only 2.5% as efficient on *E. coli* B as *E. coli* K. These phage, therefore, must have replicated in order to have been modified.

The progeny phage produced by infecting *E.*

TABLE 2. *Parental phage coat is reutilized by progeny phage*[a]

Infecting parental phage	Counts per minute				
	Associated with cells immediately after infection	Associated with cells after 120 min of growth	Which leave cells	In progeny phage	In growth medium
[^{3}H]leucine SB=0	74,000	16,700	56,900	17,600	39,300
[^{3}H]leucine SB=1	156,000	84,000	72,000	3,900	68,100

[a] Two 100-ml cultures of *E. coli* B were grown in LB broth to an OD_{600} of 0.6. One culture was infected with 0.25 ml of [^{3}H]leucine-labeled SB=0 phage; the other culture was infected with 0.25 ml of [^{3}H]leucine-labeled SB=1 phage. Both cultures were incubated for 5 min at 37 C. The cells were centrifuged, blended, and washed twice, resuspended in 400 ml of LB broth at 25 C, and grown for 3 h. Phage were purified by the method of Rossomando and Zinder (6).

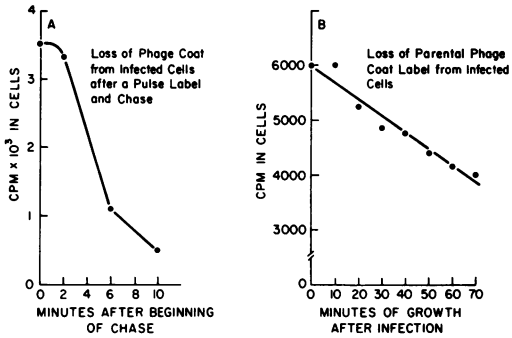

FIG. 2. *Rate of loss of phage coat from f1-infected cells. at 37 C. A, Newly synthesized phage coat. f1-infected cells were labeled with [^{14}C]lysine for 30 s. An excess of cold lysine was added to the culture, and samples of cells were assayed for f1 major coat protein at various times during the chase. This experiment was taken from Smilowitz, Lodish, and Robbins (10). B, Parentally derived phage coat. A 40-ml amount of E. coli K37 growing in LB broth was incubated with 0.05 ml of [^{3}H]lysine-labeled SB=0 phage for 8 min. The infected cells were washed twice, blended twice and grown in LB broth supplemented with unlabeled f1 phage. Samples of cells were withdrawn every 15 min, washed, and assayed for radioactivity.*

coli B cells with SB=1 phage only possesses 2.5% of the original parental phage coat label. The progeny phage produced by infecting *E. coli* B cells with SB=0 phage possess 23% of the original parental phage coat label. From this it is clear that the label found in progeny phage does not come from adventitiously bound parental labeled phage. Further, since most of the label originally present in phage coat can be recovered as phage coat, the parental phage coat must be reutilized as intact monomers and not broken down to amino acids and then reutilized. Hence, the labeled parental phage coat is indeed associated with the host cell inner membrane upon phage infection whereupon it is slowly reutilized as intact monomers by newly formed phage.

In Fig. 2 we compare the rate at which parentally derived phage coat leaves the infected cell with the rate at which infected cells lose newly synthesized phage coat at 37 C. The half-life for the loss of parental phage coat from the infected cell is seen to be 1.5 h compared to 3.5 min for newly synthesized phage coat (10).

Parental phage coat molecules as independent units. If *E. coli* B cells are doubly infected with [^{3}H]lysine-labeled SB=0 and [^{14}C]lysine-labeled SB=1, we find an equal proportion of the [^{14}C]lysine monomers and [^{3}H]lysine-labeled monomers in progeny phage. This is shown in Table 3. This result is quite unlike the 10-fold difference obtained when SB=0 and SB=1 are used separately to infect *E. coli* B cells. Hence we conclude that double infection with SB=0 and SB=1 allows SB=1 coat protein to be rescued by SB=0 genes.

We have found that f1 phage can be made dense by lactoperoxidase-catalyzed iodination while retaining viability. This technique and some of its applications will be described in detail in another publication. Here it is sufficient to state that lactoperoxidase-catalyzed iodination performed as described in Materials and Methods can be used to alter the density of the phage from 1.30 to 1.34 (Fig. 3A). [^{3}H]lysine-labeled phage was iodinated and used to infect K37 cells. The infected cells were washed and blended twice to remove non-specifically adsorbed phage (see Materials and Methods) and allowed to grow for varying lengths of time at 25 C. Progeny phage were collected after 30 min and 120 min of growth and subjected to density gradient centrifugation. Figure 3B and 3C show that the radioactivity that was originally present in the phage coat of dense parental phage is recovered in fully light progeny phage. We conclude that progeny phage contain

TABLE 3. *Rescue of SB=1 phage coat by superinfection with SB=0 phage*[a]

Superinfection	Counts per minute				
	Associated with cells immediately after infection	Associated with cells after 120 min of growth	Which leave the cells	In progeny phage	In growth medium
Infecting parental phage					
[^{3}H]lysine SB=0	461,200	152,100	309,100	133,000	176,100
[^{3}H]lysine SB=1	547,500	222,000	325,000	13,300	311,700
Mixed infection					
[^{3}H]lysine SB=0	204,200	59,700	144,300	42,000	102,300
+ [^{14}C]lysine SB=1	43,600	22,800	20,800	7,700	13,100

[a] Three cultures of *E. coli* B were grown in LB broth to an OD of 0.6. One culture was infected with 0.2 ml of [^{3}H]lysine-labeled SB=1 phage. A third culture was infected simultaneously with 0.1 ml of [^{3}H]lysine-labeled SB=0 and 0.2 ml of [^{14}C]lysine-labeled SB=1 phage. All three cultures were incubated for 5 min at 37 C. The cells were centrifuged, blended and washed twice, resuspended in 400 ml of LB broth at 25 C, and grown for 2 h at 25 C. Phage were purified by the method of Rossomando and Zinder (6).

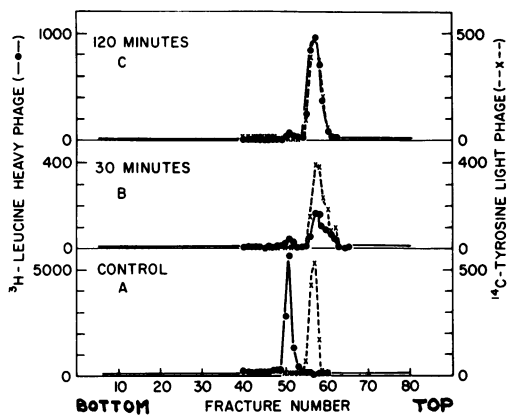

FIG. 3. *Parental phage coat monomers are not conserved when transferred to progeny. 10^{12} tritiated leucine-labeled SB=0 phage were iodinated as described in Materials and Methods. These repurified phage (O) were run on CsCl density gradients by the method of Rossomando and Zinder (6) with ^{14}C light marker phage. The results are shown in panel A. The same repurified phage were used to infect LB broth-grown E. coli K37 for 8 min at 37 C. The cells were washed twice, blended twice, and resuspended in a large volume of LB broth and grown at 25 C. After 30 min (panel B) and 120 min (panel C) of growth, samples were harvested and centrifuged. Phage were precipitated from the supernatants by the method of Rossomando and Zinder (6). The concentrated phage (●) were then run on CSCl density gradients; ^{14}C light marker phage were added to each gradient (×).*

less than 15% of the coat protein monomers derived from heavy infecting parental phage. This calculation assumes that all the phage coat monomers are saturated with iodine under these conditions, a contention that has been shown to be true and will be discussed in a separate publication. Thus the parental phage coat monomers are not conserved as a unit.

It should be noted that, after 2 h of growth at 25 C, only 30% of the cell-associated, iodinated, labeled parental phage coat has left the cell. All of the radioactivity which has left the cell can be recovered in progeny phage. When non-iodinated, labeled phage are used, 60 to 70% of the cell-associated parental phage coat leaves the cell after 2 h of growth at 25 C. However, only half of the labeled phage coat which leaves the cell can be found in phage. Hence we can conclude that although iodinated monomers are not discriminated against in phage assembly, they do not leave the cell when they are not packaged into phage.

DISCUSSION

I have shown in this paper that the major f1 coat protein of infecting parental phage can associate with the host cell inner membrane upon phage infection. Previously we demonstrated that newly synthesized major f1 coat protein is found associated with the host cell inner membrane in f1-infected cells (9). The mechanisms by which both of these processes occur are not understood, but it is clear that both take place in the absence of phage DNA synthesis. The major f1 coat protein, that is synthesized in nonpermissive *E. coli* infected with an amber mutant in gene 5 (where phage single strand synthesis is blocked), does associate with the host cell inner membrane; the major f1 coat protein of infecting parental phage is associated with the host cell particulate fraction of a colicin-tolerant mutant that does not allow phage DNA penetration and replica-

tion. Both processes require energy.

Although there are similarities in the process by which newly synthesized phage coat and parental phage coat associate with the host cell envelope, there are differences as well. In all likelihood, newly synthesized phage coat is made on cellular polysomes; there is as yet no evidence for a mechanism that transfers newly synthesized phage coat to the host cell envelope. Alternatively, parental phage are brought in apposition to the cell envelope by F pili; there, the uncoating process occurs in the absence of DNA penetration and protein synthesis.

After the phage coat associates with the host cell inner membrane, it is packaged into new phage and released from the cell (9). This occurs in both the case of the parental and the newly made coat protein, but with very different kinetics and efficiency. Eighty percent of the radioactivity in newly synthesized phage major coat protein leaves the cell in phage by 6 min after it is synthesized; 75% of the radioactively derived parental phage coat leaves the cell 2 h after phage infection and only one-third of the counts per minute are found in phage. The remaining two-thirds are recoverable from the growth medium as phage coat protein molecules. The fact that all of the radioactivity derived from parental phage can be recovered as phage coat argues strongly that the parental phage coat which associates with the host cell is not broken down to amino acids and reutilized in progeny phage but rather is reutilized as intact phage coat. The different rates at which parental phage coat and newly synthesized phage coat leave the infected cell suggests the existence of different mechanisms by which they are utilized in phage production.

Our findings allow us to say something about the mechanism by which parentally derived phage coat is packaged into phage. We have shown that parental phage coat from defective phage can be rescued by normal, superinfecting phage. We also have demonstrated that less than 15% of the coat protein monomers of an infecting phage are reutilized by any single progeny phage. Taken in concert, these findings support the notion that parental phage coat molecules act either as independent units or as small aggregates when packaged into nascent phage.

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