Localization of Membrane Protein Synthesized After Infection with Bacteriophage T4

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The synthesis of membrane protein after infection with bacteriophage T4 was examined. Protein constituents of both the cytoplasmic and outer membrane are made during the infective cycle. In addition, newly synthesized membrane protein is found in material which has a buoyant density greater than that of either of the two host membrane fractions. Polyacrylamide gel analyses and solubilization studies using the detergent Sarkosyl indicate that synthesis of most of the membrane proteins made during the first 5 min of infection is directed by bacterial genes. New membrane proteins synthesized at times greater than 6 min after infection appear to be distinct from those of the host, and new proteins of the outer membrane are different from those of the inner. Proteins in the new dense membrane fraction are similar to those of the outer membrane.

It is now well documented that infection of *Escherichia coli* cells with bacteriophage T4 results in modifications of cellular membrane. The *rIIA* (6) and *rIIB* (16, 28) gene products have been found associated with membrane, and alterations in phospholipid metabolism have been described (8, 15). The possibility that T4 infection does result in membrane modifications was first suggested to account for a number of phenomena which occur shortly after infection. These include the genetic exclusion of superinfecting phage (3), increasing tolerance to infection by ghosts (2), changes in host permeability (22), the inhibition of catabolism of exogenously added deoxynucleotides (13), and the development of resistance to lysis from without (27). T4 genes have been implicated in several of these changes: the *imm* gene is necessary for full expression of tolerance to ghosts and genetic exclusion (26), resistance to lysis from without does not develop in the spackle mutant (5), and the *ac* gene must be functional if permeability to acridine dyes is to be manifested (21).

The envelope of gram-negative cells contains two distinct membrane species, the outer (L) membrane and the inner (cytoplasmic) membrane (9). Procedures for the separation of inner from outer membrane (14, 18), which are based on the different buoyant densities of the two, and for the selective solubilization of the cytoplasmic membrane (1, 7, 19) are now available. The phenomena described above which are thought to reflect phage-induced alterations of host membrane can be explained most simply

by T4 effects on the cytoplasmic membrane only. Previous studies on separated membrane species, which have suggested that the *rIIA* and *rIIB* proteins are constituents of the cytoplasmic membrane and that synthesis of the major host proteins of the outer membrane is greatly reduced within the first 5 min of infection (6, 16), do not contradict this view. This study was undertaken, therefore, to examine the localization of membrane proteins synthesized after T4 infection. After infection, distinct new protein constituents of both the outer and inner membrane are synthesized. Also, membrane preparations are found to contain a new dense fraction, which, on the basis of protein composition, appears to be related to the outer membrane.

MATERIALS AND METHODS

The bacterial and bacteriophage strains used have been previously described, as have the procedures for growth and infection of cells and the preparation of phage lysates (4).

Media and reagents. Tryptone-NaCl broth, top agar, plates, and dilution fluid have been described previously (4). F medium consisted of a minimal salts solution (24) supplemented with 0.4% glucose and 0.05 volumes of medium A (17).

Sodium lauryl sarcosinate (Sarkosyl) was a gift of Geigy Chemical Corp., Ardsley, N.Y. Uniformly labeled L-[¹⁴C]leucine and L-[4, 5-³H]leucine were purchased from New England Nuclear Corp.

Separation of membrane. The procedure of Osborn et al. (14) was used with the few modifications described previously (7). The identification and isolation of fractions containing outer or inner membrane

have also been described (7). The dense membrane fraction was isolated by collecting that portion of the isopycnic sucrose density product below the visible band of outer membrane. The procedure of Lowry et al. (12) was used to determine the concentration of protein in these preparations.

Solubilization of the cytoplasmic membrane. Sarkosyl was employed to selectively disrupt the cytoplasmic membrane (7).

Polyacrylamide gel electrophoresis. The procedures used were identical to those described previously (7). Gels were stained by the method of Inouye and Guthrie (10). Standards for molecular weight determinations consisted of lysozyme (14,000), immunoglobulin G (IgG) light chain (23,500), ovalbumin (43,000), IgG heavy chain (50,000), bovine-serum albumin (68,000), and the dimer of ovalbumin.

RESULTS

Localization of membrane proteins synthesized after infection. Membrane proteins synthesized at various intervals during T4 infection were examined by isolating the total membrane fraction and then separating the two host membrane species. Infection was carried out under nonpermissive conditions with a DNA-negative mutant, T4amN82, to avoid complications arising from membrane-associated maturation of T4 particles (20, 23, 25). Cells were prelabeled with [14 C]leucine to determine the effectiveness of the membrane separation procedure and to indicate the positions of host membrane species in the isopycnic sucrose density gradients.

Results of an experiment in which samples of a culture were pulse labeled with [3 H]leucine for 1 to 5, 5 to 9, or 9 to 13 min after infection are shown in Fig. 1. The outer membrane of the host has a density of 1.22 g/cm³ and appears at a position approximately 0.2 from the bottom of the gradient and the cytoplasmic membrane, which has a density of 1.16 g/cm³, is present at a position approximately 0.7 from the bottom (14). During the 1- to 5-min interval, newly synthesized membrane proteins are distributed equally between outer and inner membrane (panel A). Identical results are obtained if an uninfected culture is pulse labeled for 4 min.

At later times in infection (panels B and C), new proteins in the membrane fraction continue to appear at densities characteristic of both L and cytoplasmic membrane. In addition, some protein now appears at a density greater than that of the outer membrane. This new band of membrane protein was studied further by examining proteins made during a 9-min pulse, begun 6 min postinfection, with [3 H]leucine. Proteins present in the new band accumulate during infection with amN82; as shown in Fig. 2, approximately 38% of the new membrane

FIG. 1. Separation of membrane from infected cells. Overnight *E. coli* B were diluted 1:100 in 150 ml of broth supplemented with 0.2 μ Ci of L-[14 C]leucine/ml and grown at 37 C to a concentration of 4×10^8 cells/ml. Cells were harvested and resuspended in an equal volume of prewarmed broth supplemented with 50 μ g of L-tryptophan/ml. The culture was divided into three equal portions, and each portion was infected with seven T4amN82/cell. Each portion was pulse labeled with 10 μ Ci of L-[3 H]leucine/ml for a different 4-min interval beginning 1 min after infection. At the end of the pulse period, samples were chilled by pouring over one-half volume of crushed, frozen broth. Isolation and fractionation of membrane was then carried out as described in Materials and Methods. Results of pulse labeling with L-[3 H]leucine 1 to 5, 5 to 9, and 9 to 13 min after infection are shown in panels A, B, and C, respectively. Symbols: $\bullet$, L-[14 C]leucine label; $\circ$, L-[3 H]leucine label.

protein made during the 9-min interval appears in this region. The heavy membrane fraction is also present after infection with wild-type T4; the time of its appearance and, for the first 9 min of infection, the percentage of new membrane protein it contains are similar to those observed in T4amN82 infections.

Selective solubilization of the cytoplasmic membrane of infected cells. The ionic detergent Sarkosyl, in the absence of Mg^{2+} , selectively disrupts the inner membrane of *E. coli* cells (7). Evidence that Sarkosyl acts in a similar manner on membrane from infected cells is shown in Fig. 3 and 4. Membrane isolated from cells which had been infected for 4 min was treated with Sarkosyl. Proteins solubilized by Sarkosyl, which are found at the top of the isopycnic sucrose gradient, were analyzed by polyacrylamide gel electrophoresis and compared with purified inner membrane proteins (Fig. 3). Proteins in membrane resistant to Sarkosyl disruption, which continue to band at a buoyant density of 1.22, were similarly compared to proteins from purified L membrane (Fig. 4). The principal proteins solubilized by Sarkosyl are similar to those present in the cytoplasmic membrane and, with the exception of one low-molecular-weight band present only in purified outer membrane, those of the outer membrane correspond to proteins which are not released from the membrane by detergent. These results indicate that cytoplasmic membrane, but not outer membrane, of cells which have been infected for a short time is disrupted by Sarkosyl.

FIG. 2. Localization of membrane protein synthesized 6 to 15 min after infection with T4amN82. The procedure is essentially the same as that described in Fig. 1, except that cells were grown in broth supplemented with $0.07 \mu\text{Ci}$ of $L\text{-}[^{14}\text{C}]\text{leucine/ml}$, and the pulse was carried out with $3 \mu\text{Ci}$ of $L\text{-}[^3\text{H}]\text{leucine/ml}$ 6 to 15 min postinfection. Symbols: $\bullet$, $L\text{-}[^{14}\text{C}]\text{leucine}$; $\circ$, $L\text{-}[^3\text{H}]\text{leucine}$ label.

FIG. 3. Comparison of inner membrane proteins with membrane proteins from infected cells which are solubilized by Sarkosyl. *E. coli* B was grown in 200 ml of broth at 37°C to a cell concentration of $3.6 \times 10^8/\text{ml}$. $L\text{-tryptophan}$ ($50 \mu\text{g/ml}$) was added and the culture was infected with a multiplicity of 7.5 T4amN82/cell. Four minutes after infection, the culture was poured onto one-half volume of crushed, frozen broth. The membrane fraction was isolated and incubated with 0.5% Sarkosyl for 20 min at 23°C before isopycnic sucrose density gradient centrifugation. After fractionation, the material at the top of the gradient and at the position of outer membrane was collected and concentrated. Inner and outer membrane from uninfected cells were isolated as described in Materials and Methods. The four membrane samples were digested and then run individually on 7.5% acrylamide gels containing dodecyl sodium sulfate and were cross-linked with ethylene diacrylate for 15 h at 3 mA/gel. Gels were stained with Coomassie blue. Left gel, protein ($150 \mu\text{g}$) solubilized by Sarkosyl; right gel, protein ($166 \mu\text{g}$) of cytoplasmic membrane.

Effect of Sarkosyl on protein of membrane from infected cells. It has been suggested that proteins added to membrane after infection are less strongly bound in membrane than pre-existing host proteins (28). The selective effect

FIG. 4. Comparison of outer membrane proteins with those from infected cells which are not solubilized by Sarkosyl. Procedures are described in the legend for Fig. 3. Left gel, proteins (300 μ g) from infected cells which are not solubilized by Sarkosyl; right gel, proteins (300 μ g) of outer membrane.

of Sarkosyl was employed to study this problem with respect to new proteins of the outer membrane (Fig. 5). (The panels in Fig. 5 can be compared with those in Fig. 1 to note the specific effects of Sarkosyl on membrane from infected cells.) Two conclusions can be drawn. If only that protein made prior to infection is examined, the detergent is seen to disrupt only cytoplasmic membrane and membrane of intermediate density, which consists of unfractionated inner and outer membrane (14). This observation supports and extends the results shown in Fig. 3 and 4. Pre-existing outer membrane is resistant to Sarkosyl disruption even after 13 min of infection with *amN82*.

A very different result was found for those membrane proteins synthesized after infection which banded at a density similar to that of outer membrane; these displayed an increasing sensitivity to solubilization by Sarkosyl with

time. As seen in panel A, new outer membrane proteins synthesized 1 to 5 min after infection appear to be integral constituents of the L membrane, as judged by their resistance to detergent treatment. In contrast, 41% of the outer membrane protein made 5 to 9 min postinfection (panel B) and all of the membrane protein made at times greater than 9 min after infection (panel C) are solubilized by Sarkosyl. The increase in detergent sensitivity is not the result of an overall change in membrane which occurs during the course of infection. When membrane proteins of cells labeled from 3 to 15 min after infection are examined, 37% of the label associated with outer membrane is resistant to solubilization by Sarkosyl (data not shown). On the basis of these studies, two classes of new outer membrane protein, detergent resistant and detergent sensitive, can therefore be distinguished. Also, none of the pulse-labeled protein which appeared at densities greater than that of the outer membrane (Fig. 1 and 2) was resistant to disruption by Sarkosyl.

Protein constituents of the cytoplasmic, outer and dense membrane species of infected cells. Membrane was isolated from cells which

FIG. 5. Effect of Sarkosyl on membrane from infected cells. Conditions of growth, infection, and labeling with radioactive leucine were identical to those described in the legend for Fig. 1. Membrane fractions (1 ml) each received 0.1 ml of a 5% Sarkosyl solution and were incubated for 20 min at 23 C prior to fractionation. Symbols and identification of panels are the same as those used in Fig. 1.

had been grown in [^{14}C]leucine, transferred to nonradioactive medium, infected with T4amN82, and labeled with [^3H]leucine for the 1- to 5- or 6- to 15-min interval after infection. This material was separated into inner, outer, and dense membrane, and the protein present in each membrane species was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 6). Previous studies have shown that T4 infection does not result in alterations in pre-existing total membrane protein (28; Wulff and Earhart, unpublished observation); it was therefore not surprising that, after 15 min of infection, no significant changes in the previously labeled host proteins of outer or inner membrane were observed. The pre-existing proteins in cytoplasmic membrane preparations isolated 5 (Fig. 6A) or 15 (Fig. 6D) min after infection are similar to one another and to the inner membrane proteins of uninfected cells (Fig. 3 of reference 7). Evidence that protein components of the outer membrane are also essentially unaltered by infection can be obtained by comparing Fig. 6B and F with Fig. 3 in reference 7. It is therefore possible to

directly compare the [^3H]leucine profiles with the [^{14}C]leucine profiles to determine the extent of similarity between proteins made before and after infection.

Many of the membrane proteins made 1 to 5 min after infection are similar to those present in uninfected host cells (Fig. 6A and B). Significant differences occur primarily in the molecular weight range of 20,000 and below. Fractions 65 to 90 in Fig. 6A include proteins whose molecular weights range from 19,500 to 7,300; in Fig. 6B, fractions 60 to 82 encompass proteins of molecular weights 20,000 to 8,300. Of the pre-existing protein in membrane isolated after 5 min of infection, 5% was found with the dense fraction, 46% was found in the outer membrane, and 49% was found in the cytoplasmic membrane. Membrane proteins made 1 to 5 min postinfection were distributed among the three membrane species as follows: dense, 7.7%; outer, 41%; and inner, 52%.

Membrane proteins synthesized 6 to 15 min after infection appear to be quite distinct from *E. coli* membrane proteins (Fig. 6D and E), and the ensemble of new proteins associated with

FIG. 6. Polyacrylamide gel electropherograms of proteins in membrane species isolated from infected cells. A 250-ml culture of *E. coli* B was grown at 37°C in F medium supplemented with 0.04 μCi of L-[^{14}C]leucine/ml. At a density of 4×10^8 cells/ml, the cells were harvested and resuspended in an equal volume of prewarmed, nonradioactive medium containing 50 μg of L-tryptophan per ml. The culture was divided into two equal portions, and each portion was infected with T4amN82 at a multiplicity of 5. The infected cells of one portion (infected culture 1) were labeled with L-[^3H]leucine (0.8 μCi /ml) 1 to 5 min after infection and the other portion (infected culture 2) was labeled with L-[^3H]leucine 6 to 15 min after infection. Infected cultures were poured onto one-half volume of frozen, crushed minimal medium at the end of the labeling period. Membranes from each portion were isolated, separated, concentrated, digested, and analyzed on continuous sodium dodecyl sulfate polyacrylamide gels. Symbols: —, L-[^{14}C]leucine label; —, L-[^3H]leucine label. Panels A to C: proteins of the inner, outer, and dense membrane species, respectively, from infected culture 1. Panels D to F: proteins of the inner, outer, and dense membrane species, respectively, from infected culture 2.

the inner membrane is different from the complement of new proteins in the outer membrane. Thirty-eight percent of the newly made membrane protein is found with the cytoplasmic membrane; of this, 46% has a molecular weight of less than 13,000 (Fig. 6D). In contrast, the most prevalent new proteins in the outer membrane have molecular weights in the 35,000 to 40,000 range (fractions 45 to 51). Only 21% of the membrane protein made 6 to 15 min after infection was found in this outer membrane; 41% was found in the dense fraction. The dense, outer, and inner membrane preparations contained 8%, 36%, and 57% of the pre-existing membrane protein, respectively.

On the basis of its protein constituents, the dense membrane fraction appears to be related to outer membrane; both the pre-existing and newly synthesized proteins in the dense fraction are similar to those of the outer membrane (Fig. 6C and F). The procedure used to isolate the dense fraction probably results in some contamination of this material by outer membrane. This is revealed most clearly in samples taken after the first 5 min of infection. Sucrose gradient results indicate no material is present in this dense fraction (Fig. 1A), but 7.7% of the newly synthesized protein is recovered in the dense fraction isolated for analysis by gel electrophoresis. However, this level of contamination of dense by outer membrane cannot account for the similarities observed between the two membrane species when they are separated after 15 min of infection (Fig. 6E and F).

DISCUSSION

Three principal findings emerge from these initial studies of membranes isolated from T4-infected cells. The first simply extends the previous observation that Sarkosyl treatment of membrane from uninfected cells solubilizes only cytoplasmic membrane and mixtures of outer and cytoplasmic membrane. Membrane proteins synthesized prior to infection are shown to retain their characteristic sensitivity to detergent even after 13 min of infection (Fig. 3 to 5). No changes have been detected in the electrophoretic profiles of pre-existing proteins present in total membrane preparations (28; Wulff and Earhart, unpublished observation) or in separated inner and outer membrane preparations (Fig. 6) as a result of infection. Taken together, these results suggest that the basic structure of inner and outer membrane is not greatly altered during the early stages of infection.

The second observation is that membrane protein synthesized after infection with

T4amN82 appears to be of two temporal classes. Polyacrylamide gel electropherograms show that much of the protein made during the 1- to 5-min interval after infection is similar to that specified by host genes (Fig. 6). For both inner and outer membrane, obvious differences occur primarily among proteins of low molecular weight. We do not believe that the differences between the profiles of pre-existing and newly synthesized membrane protein arise from the different labeling periods employed. Others (28) have found that gel profiles of membrane protein from uninfected cells are identical when labeling is carried out for 0.2 or 2.0 generations. Similarly, when *E. coli* B is growing at 30°C with a doubling time of 53 min in a glucose-salts medium, proteins from membrane isolated after a 5-min labeling period display an identical profile to that obtained from cells labeled for 4 generations (Wulff and Earhart, unpublished observation).

Proteins synthesized 6 to 15 min after infection are quite distinct from host protein (Fig. 6). T4 appears to direct the synthesis of unique new protein constituents of both the outer and inner membrane. Comparison of the 1- to 5- with the 6- to 15-min profiles of new protein suggests that synthesis of bacterial membrane protein continues for the first several minutes of infection and then, by 6 min after infection, is either greatly reduced in rate or completely shut off. Inhibition of synthesis of the major proteins of the outer membrane has been reported to take place during the first 5 min of infection (16), but little else is known concerning the cessation of synthesis of host-specified membrane proteins. The selective effect of Sarkosyl on newly synthesized proteins of the outer membrane provides indirect evidence that host membrane protein synthesis is completely stopped during infection. Most new proteins of the outer membrane which are synthesized early in infection, and therefore probably primarily host specified, are resistant to solubilization by Sarkosyl. In contrast, all new membrane protein synthesized 9 min or later during infection is totally sensitive to detergent disruption (Fig. 5).

The gel electropherograms also provide evidence that there may be two classes of phage-specified membrane proteins synthesized during amN82 infection. The *rIIA* and *rIIB* proteins are first detected approximately 5 to 8 min after infection, and their synthesis proceeds for at least the first 13 min of infection (6, 28). This class of membrane protein is best represented in our data by the new major proteins of the outer membrane (fractions 41 to 50, Fig. 6E). Tentative evidence for a second class of protein, which is synthesized primarily during the first 5 min of

infection, is seen in the broad peak of protein in fractions 65 to 75 of the cytoplasmic membrane (Fig. 6A) and in the protein in fractions 60 to 68 of the outer membrane (Fig. 6B). Definitive studies on membrane proteins made early in infection will require that host protein synthesis be blocked at the time of infection.

It is possible that no membrane proteins synthesized under the direction of the T4 genome are resistant to solubilization by Sarkosyl; that is, outer membrane protein made after infection which is not disrupted by Sarkosyl may represent residual synthesis of bacterial protein. Some previous data has been interpreted as evidence that T4 proteins are not as firmly associated with membrane as the membrane proteins of uninfected cells (28). Gel profiles of *E. coli* protein were found to vary with the temperature of disaggregation, whereas T4 proteins yielded identical profiles after solubilization at either 60 or 100 C. However, recent studies of *E. coli* envelope proteins have led to the suggestions that variations in gel profiles with differing conditions of solubilization may result from the presence of glycoprotein or lipoprotein (11), or, as now seems more probable, variations in the charge/mass ratios of the major proteins of the outer membrane (29).

The third point to be emphasized is the presence of protein in membrane isolated from infected cells which bands at a different density than either of the two species of host membrane. The membrane separation procedure employed is sometimes found to be less effective when membrane from infected cells is used. The reason for this is not known. However, use of prelabeled host cells precludes the possibility that the new peak is an artifact. The new peak begins to appear at approximately 6 min postinfection, is sensitive to Sarkosyl disruption, and accounts for approximately 38% of the total membrane protein made from 6 to 15 min after infection with T4amN82. Analysis of the protein constituents of this fraction indicates that it is related to outer membrane. Contamination of this membrane species by outer membrane occurs, but the extent of this contamination is not sufficient to account for the similarity in profiles of newly synthesized protein observed in Fig. 6E and F. From the available data, it is not possible to determine if any proteins are unique to the dense fraction. If this is found to be the case, classification of T4 membrane proteins as either constituents of outer or inner membrane would be an obvious oversimplification.

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LITERATURE CITED

1. De Pamphilis, M. L., and J. Adler. 1971. Attachment of flagellar basal bodies to the cell envelope: specific attachment to the outer, lipopolysaccharide membrane and the cytoplasmic membrane. *J. Bacteriol.* 105:396-407.
2. Duckworth, D. H. 1971. Inhibition of T4 bacteriophage multiplication by superinfecting ghosts and the development of tolerance after bacteriophage infection. *J. Virol.* 7:8-14.
3. Dulbecco, R. 1952. Mutual exclusion between related phages. *J. Bacteriol.* 63:209-217.
4. Earhart, C. F. 1970. The association of host and phage DNA with the membrane of *Escherichia coli*. *Virology* 42:429-436.
5. Emrich, J. 1968. Lysis of T4-infected bacteria in the absence of lysozyme. *Virology* 35:158-165.
6. Ennis, H. L., and K. D. Kievitt. 1973. Association of the rIIA protein with the bacterial membrane. *Proc. Nat. Acad. Sci. U.S.A.* 70:1468-1472.
7. Filip, C., G. Fletcher, J. L. Wulff, and C. F. Earhart. 1973. Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium-lauryl sarcosinate. *J. Bacteriol.* 115:717-722.
8. Furrow, M. H., and L. I. Pizer. 1968. Phospholipid synthesis in *Escherichia coli* infected with T4 bacteriophages. *J. Virol.* 2:594-605.
9. Glauert, A. M., and M. J. Thornley. 1969. The topography of the bacterial cell wall. *Annu. Rev. Microbiol.* 23:159-198.
10. Inouye, M., and J. P. Guthrie. 1969. A mutation which changes a membrane protein of *E. coli*. *Proc. Nat. Acad. Sci. U.S.A.* 64:957-961.
11. Inouye, M., and M. L. Yee. 1973. Homogeneity of envelope proteins of *Escherichia coli* separated by gel electrophoresis in sodium dodecyl sulfate. *J. Bacteriol.* 113:304-312.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
13. Munch-Petersen, A., and M. Schwartz. 1972. Inhibition of the catabolism of deoxyribonucleosides in *Escherichia coli* after infection by T4 phage. *Eur. J. Biochem.* 27:443-447.
14. Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson. 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. Isolation and characterization of cytoplasmic and outer membrane. *J. Biol. Chem.* 247:3962-3972.
15. Peterson, R. H. F., and C. S. Buller. 1969. Phospholipid metabolism in T4 bacteriophage-infected *Escherichia coli* K-12 (λ). *J. Virol.* 3:463-468.
16. Peterson, R. F., K. D. Kievitt, and H. L. Ennis. 1972. Membrane protein synthesis after infection of *Escherichia coli* B with phage T4: the rIIB protein. *Virology* 50:520-527.
17. Rothfield, L., M. J. Osborn, and B. L. Horecker. 1964. Biosynthesis of bacterial lipopolysaccharide. II. Incorporation of glucose and galactose catalyzed by particulate and soluble enzymes in *Salmonella*. *J. Biol. Chem.* 239:2788-2795.
18. Schnaitman, C. A. 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. *J. Bacteriol.* 104:890-901.
19. Schnaitman, C. A. 1971. Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. *J. Bacteriol.* 108:545-552.
20. Showe, M. K., and L. W. Black. 1973. Assembly core of

- bacteriophage T4: an intermediate in head formation. *Nature N. Biol.* **242**:70-75.
21. Silver, S. 1967. Acridine sensitivity of bacteriophage T2: a virus gene affecting cell permeability. *J. Mol. Biol.* **29**:191-202.
 22. Silver, S., E. Levine, and P. M. Spielman. 1968. Cation fluxes and permeability changes accompanying bacteriophage infection of *Escherichia coli*. *J. Virol.* **2**:763-771.
 23. Simon, L. D. 1972. Infection of *Escherichia coli* by T2 and T4 bacteriophages as seen in the electron microscope: T4 head morphogenesis. *Proc. Nat. Acad. Sci. U.S.A.* **69**:907-911.
 24. Siström, S. R. 1958. On the physical state of the intracellularly accumulated substrates of β -galactoside-permease in *Escherichia coli*. *Biochim. Biophys. Acta* **29**:579-587.
 25. Takano, T., and T. Kakefuda. 1972. Involvement of a bacterial factor in morphogenesis of bacteriophage capsid. *Nature N. Biol.* **239**:34-37.
 26. Vallee, M., and J. B. Cornett. 1972. A new gene of bacteriophage T4 determining immunity against superinfecting ghosts and phage in T4-infected *Escherichia coli*. *Virology* **48**:777-784.
 27. Visconti, N. 1953. Resistance to lysis from without in bacteria infected with T2 bacteriophage. *J. Bacteriol.* **66**:247-253.
 28. Weintraub, S. B., and F. R. Frankel. 1972. Identification of the T4rIIb gene product as a membrane protein. *J. Mol. Biol.* **70**:589-615.
 29. Wu, H. C. 1972. Isolation and characterization of an *Escherichia coli* mutant with alteration in the outer membrane proteins of the cell envelope. *Biochim. Biophys. Acta* **290**:274-289.