

Membrane fusion during infection of *Escherichia coli* cells by phage T4

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Phage T4 infection of *Escherichia coli* was studied by thin-section and freeze-fracture electron microscopy. It was found that phage T4 induces the formation of a bridge between the outer and inner membranes of *E. coli*. A membrane fusion during the infection is suggested.

Phage infection; Membrane fusion; Electron microscopy; DNA transport; *Escherichia coli*

1. INTRODUCTION

In Gram-negative cells, high molecular weight compounds might be transported through the sites of adhesion between the outer and inner membranes [1], or through the sluice structures suggested recently [2]. However, the hypothetical direct membrane contacts or fusions that participate in such a transport have been visualized only after special treatment of cells [3]. Bacteriophage T4 also attaches to the adhesion sites or induces their formation [4]. We have studied the membranes of *Escherichia coli* during T4 infection by freeze-fracturing and thin-sectioning techniques. It was found that, under our conditions, phage T4 core does not penetrate the outer membrane as has been assumed earlier [5,6], but initiates membrane fusion. We suggest that the transmembrane electrical potential (or electrochemical gradient of cations) necessary for cell infection [7–9] acts by providing the appropriate conditions for membrane fusion. A new model of phage T4 infection of *E. coli* is presented.

2. MATERIALS AND METHODS

Phage T4B was grown and purified as described previously [10]. *E. coli* B was grown to 5×10^8 cells/ml, harvested, washed once and resuspended in fresh nutrient broth. The multiplicity of infection was 200. Phages and cells were incubated together for 2 min at 20°C and then concentrated by centrifugation at room temperature at $8000 \times g$ for 2 min. The time from the beginning of the infection to the cryo- or chemical fixation was about 5 min. Ultrarapid plunge-freezing without cryoprotectants and subsequent fracturing were described previously [3]. For chemical fixation, cells were treated with glutaraldehyde, osmium tetroxide, tannic acid and again osmium tetroxide (GOTO-fixation). As was shown earlier this sequence of fixation prevents

possible artifacts of tannic acid action [11]. In our experiments 1% fixative solvents in 100 mM phosphate buffer (pH 7.4), and one hour fixation with each solvent were used. After every step of fixation the cells were washed 3 times with phosphate buffer. For energy depletion [7–9] *E. coli* cells were treated with 60 μ M CCCP at 37°C for 20 min or by 30 mM cyanide for 10 min and then by 30 mM arsenate for 10 min at 37°C.

3. RESULTS AND DISCUSSION

Fixation under our conditions reveals different stages of infection. It appears likely, that the invagination of the outer membrane by phage and the formation of contacts between membranes are the first stages of phage penetration through the cell wall (Fig. 1). The elimination or redistribution of intra membrane particles in contacting regions (Fig. 1A) might precede membrane fusion, similar to that in eukaryotic cells [12]. We suggest, that the intermembrane bridges observed in ultrathin sections (Fig. 2) are the results of such a membrane fusion. The phage core seems to be extended into the central part of the bridge and may have a dark or light central part (Fig. 2A,B). The light core is more typical for phages with empty heads, suggesting that the electron density of that region depends on the existence of phage DNA.

The measurements indicate that the whole diameter of the bridge is about 120–130 Å (Fig. 2C) which is much more than the diameter of the phage T4 core (about 90 Å) [13]. On the other hand, the diameter of the central part of the bridge is about 45–55 Å. The width of the electron transparent lines composing the bridge (30–40 Å) is typical for the hydrophobic region of membranes. According to our measurements it is likely that the core proteins extend into the unstained and possible hydrophobic part of the bridge. (Fig. 3). Therefore, we suggest that there might be an interaction of core proteins with membrane lipids within the bridge, which is in agreement with hydrophobic properties of the core tip [14].

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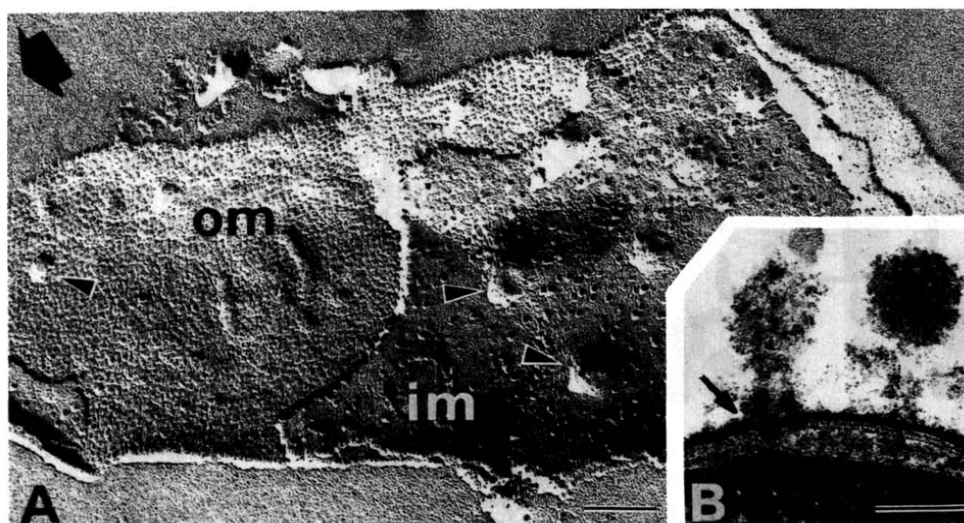


Fig. 1. Invagination of membranes of *Escherichia coli* cells induced by phage T4. On fracture faces (A) of outer (OM) and inner membranes (IM) the invaginations deprived of intramembrane particles (arrowheads) are observed (the concave fracture surface is presented). The invaginations (arrow) presented also on thin-sections (B). The black arrows in freeze-fracture micrographs indicate the direction of shadowing. Bars represent 0.2 μm .

About 1–5% of phages form periplasmic vesicles (Fig. 4) instead of intermembrane bridges. The fracture faces of the vesicles and their contact sites with the inner membrane are usually deprived of intramembrane particles (Fig. 4A,B). There are two types of periplasmic vesicles. The vesicles of the first type have close contact with the inner membrane (Fig. 4C). In the second type of vesicle a semifusion between membranes and one membrane elimination [12] is observed (Fig. 4D). So it

is possible that the membranes approached by the phage are ready for fusion but fusion does not occur because of an unknown defect.

The fusion membranes and the formation of intermembrane bridges does not occur after treatment of cells with CCCP, cyanide or arsenate before infection. This correlates with the requirement of a membrane potential for cell infection by phage T4 [7–9]. Meanwhile, in poisoned cells the formation of periplasmic

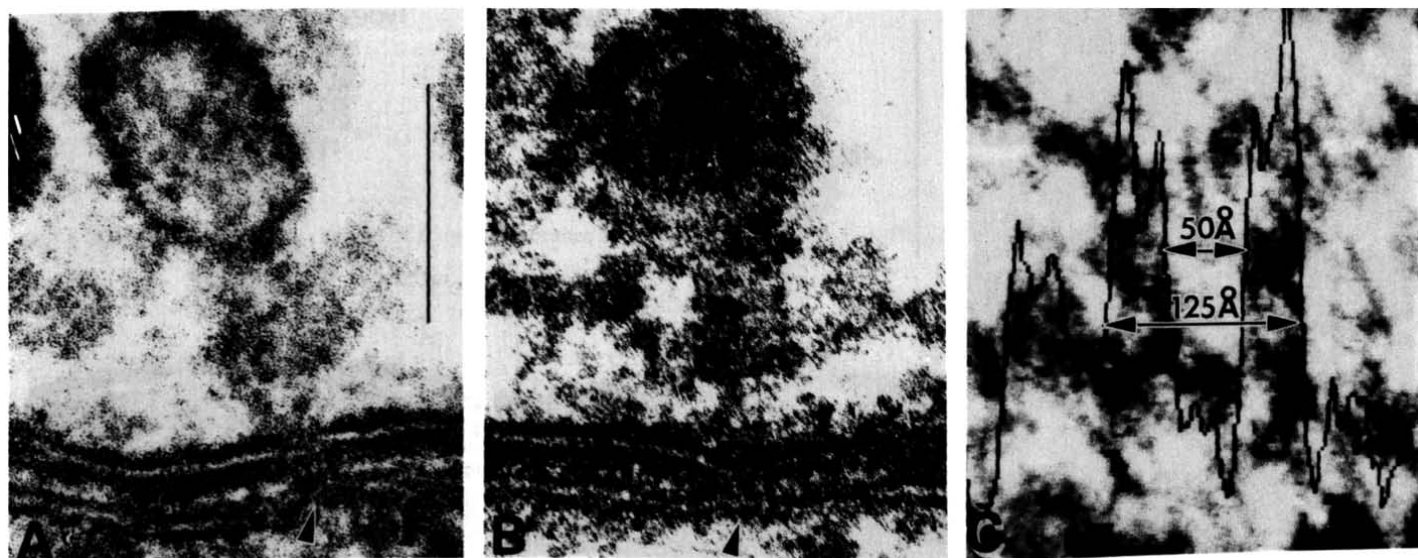


Fig. 2. Intermembrane bridges formed by phage T4 (arrowheads) with light (A) and dark (B) central part. The average density distribution across the bridge from figure B is presented on C (the negative is given). We would like to note that bridges are always visible as closed (but not as channel) because sections much thicker (about 500 Å) than the bridge.

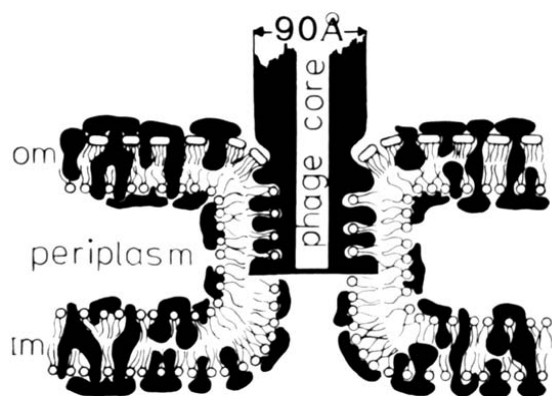


Fig. 3. The schematical illustration of a possible hydrophobic interaction between phage core proteins and the lipid bilayer in the bridge.

vesicles is still observed. It is possible that normal infection may be prevented by an increase of the intermembrane distance in poisoned cells. The changes of the intermembrane distance have been studied on rapidly frozen and fractured cells which lack possible artifacts as a result of chemical fixation. As was found, the distance between membranes for untreated cells was about 120 Å, which correlates well with other measurements [5]. However, in poisoned cells the distance was about 2–3 times larger (220–330 Å). This change of periplasmic space might result from the cytoplasm contraction after energy depletion of cells and subsequent loss of the osmotically active K^+ [15]. The intermem-

brane distance was usually decreased after infection of poisoned cells by phages.

E. coli membrane penetration by phage T4 core is presented in Fig. 5. According to the scheme, the distance between membranes is of great importance for their fusion. We suggest that in poisoned cells the phage core penetrates only through the outer membrane and injects the phage DNA into the periplasmic space as observed by some authors [9]. Similar penetration of the outer membrane by the core possibly takes place also in models of the outer membrane studied by Furukawa et al. [6].

It is known that the early stages of infection are characterized by the formation of a membrane channel and by ion leakage of membranes. A few minutes later the ion leakage decreases as a result of a sealing reaction [16]. According to our scheme the membrane leakage is followed by the penetration of the core through the adjacent membranes, subsequent membrane fusion, and hole formation. The sealing reaction could result from the formation of the bridge where the close hydrophobic interaction between the membrane lipids and the phage core proteins occurs. In such a structure the only way for ion leakage is the phage core channel that is possibly sealed with specialized proteins (not shown).

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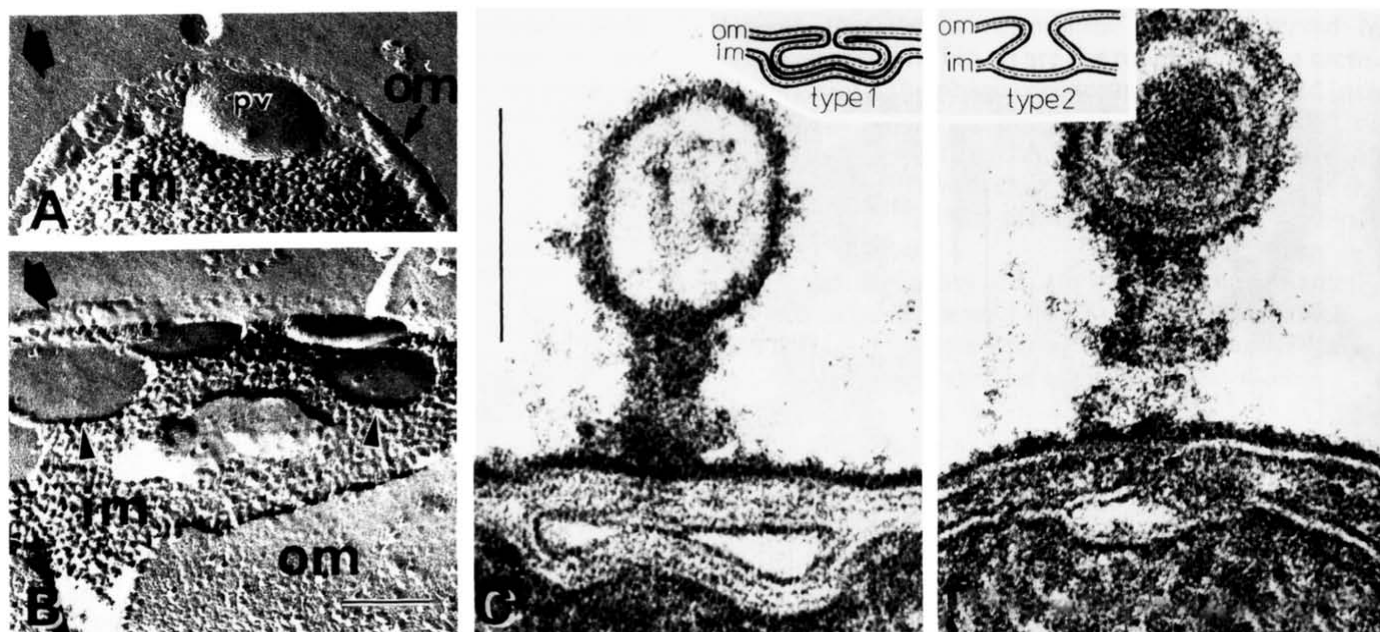


Fig. 4. Periplasmic vesicles (PV) induced by phage T4 (A). Arrowheads indicate the regions deprived of particles on convex fracture faces of inner membrane (B). Thin-sectioning (C,D) reveals two types of vesicles. The vesicles do not always contain an electron dense material, even when the phage heads are empty. It is likely that injected T4 DNA can be lost by the vesicles through the opening of the invagination.

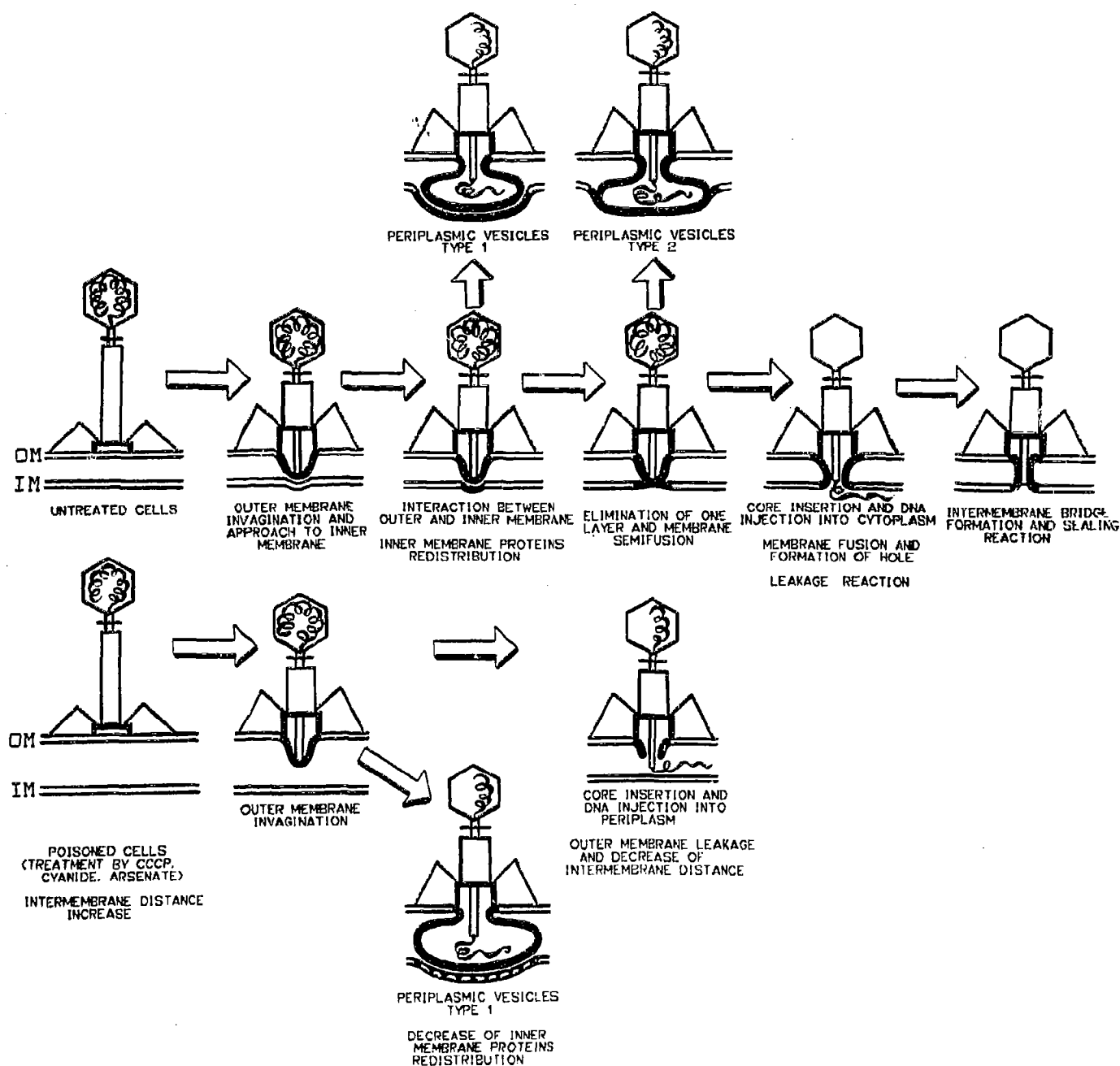


Fig. 5. A scheme of structural changes of *E. coli* membranes during infection by phage T4. Phage adsorption and contraction is in accordance with Simon and Anderson [5].

REFERENCES

- [1] Bayer, M.E. (1979) in: Bacterial Outer Membranes: Biogenesis and Function (Inouye, M., ed.) Wiley, New York, pp. 167-202.
- [2] Skulachev, V. (1991) Biosci. Rep. in press.
- [3] Borovjagin, V.L., Sabelnikov, A.G., Tarahovsky, Y.S. and Vasilenko, I.A. (1987) J. Membr. Biol. 100, 229-242.
- [4] Bayer, M.E. (1968) J. Virol. 2, 346-356.
- [5] Simon, L.D. and Anderson, T.F. (1967) Virology 32, 279-297.
- [6] Furukawa, H., Kuroiwa, T. and Mizushima, S. (1983) J. Bacteriol. 154, 938-945.
- [7] Labedan, B. and Goldberg, E.B. (1979) Proc. Natl. Acad. Sci. USA 76, 4669-4674.
- [8] Labedan, B., Heller, K.B., Jasaitis, A.A., Wilson, T.H. and Goldberg, E.B. (1980) Biochem. Biophys. Res. Commun. 93, 625-630.
- [9] Kalasauskaitė, E., Grinius, L., Kadišaitė, D. and Jasaitis, A. (1980) FEBS Lett. 117, 232-236.
- [10] Shilnikov, G.V., Khusainov, A.A., Sarvazyan, A.P. and Williams, A.R. (1986) Ultrasound Med. Biol. 12, 511-517.
- [11] Asami, K. (1986) J. Ultrastruct. Mol. Struct. Res. 95, 38-46.

- [12] Pinto da Silva, P. (1988) in: *Molecular Mechanisms of Membrane Fusion* (Ohki, S., Doyle, D., Flanagan, T.D., Hui, S.W. and Mayhew, E., eds.) Plenum, New York, pp. 521-530.
- [13] Eiserling, F.A. (1983) in: *Bacteriophage T4* (Mathews, C.K., Kutter, E.M., Mosig, G. and Berget, P.B.) American Society for Microbiology, Washington, pp. 11-24.
- [14] Furukawa, H. and Mizushima, S. (1982) *J. Bacteriol.* 150, 916-924.
- [15] Epstein, W. and Laimins, L. (1980) *Trends Biochem. Sci.* 5, 21-23.
- [16] Letellier, L. and Boulanger, P. (1989) *Biochimie* 71, 167-174.