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Control of Membrane Morphogenesis in Bacteriophage PM2

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The regulation of membrane formation in bacteriophage PM2 serves as a simple model for changes in membrane structure in eukaryotic cells. Prior to Pseudomonas host lysis, wild-type virions mature to an icosahedral morphology at the inner face of the cytoplasmic membrane. The preliminary characterization of two temperature-sensitive mutants of PM2 is described.

In cells infected at the restrictive temperature with ts 1, an abundance of "empty" virus-size membrane vesicles are seen. Synthesis of DNA is also reduced in ts I infected cells. The preponderance of vesicles is not seen in cells infected with wild-type virus or with ts 1 at the permissive temperature. The "empty" appearance of the viral membranes suggests that viral DNA is not encapsulated.

The major viral capsid protein (MW 26,000) is located just outside the viral membrane and normally sediments with host and virus membranes. This protein made by mutant ts 5 does not pellet with these membranes; instead, large amounts of capsid protein can be precipitated from the supernatant with TCA. Compared to cells infected with wild type virus, cells infected with ts 5 at the restrictive temperature produce inside the cell an abundance of virus-size membrane vesicles.

Taken together, these results with viral mutants suggest that formation of a viral membrane of the proper size does not require a DNA core around which to form, or an outer scaffolding of coat protein against which to form a spherical bilayer.

Key words: membrane, PM2, temperature-sensitive mutant, DNA core, viral coat protein

INTRODUCTION

The factors that determine and maintain the morphology of a biological membrane are poorly understood. This conference has demonstrated that at the gross level of the whole mammalian cell, membrane morphology or cell shape may be controlled to some extent by a cytoskeleton composed of actin-like microfilaments and microtubules. Still unexplained in cells, however, is the formation and control at the finer level of microvillus and ruffle formation. Because of the complexity of animal cell membranes, I have chosen to study the control of membrane morphogenesis in bacteriophage PM2.

Abbreviations: VSM (virus-storage medium), MTS(1) made 1M in NaCl; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; CAA, 0.2% casein hydrolysate (Sigma); TCA, trichloracetic acid

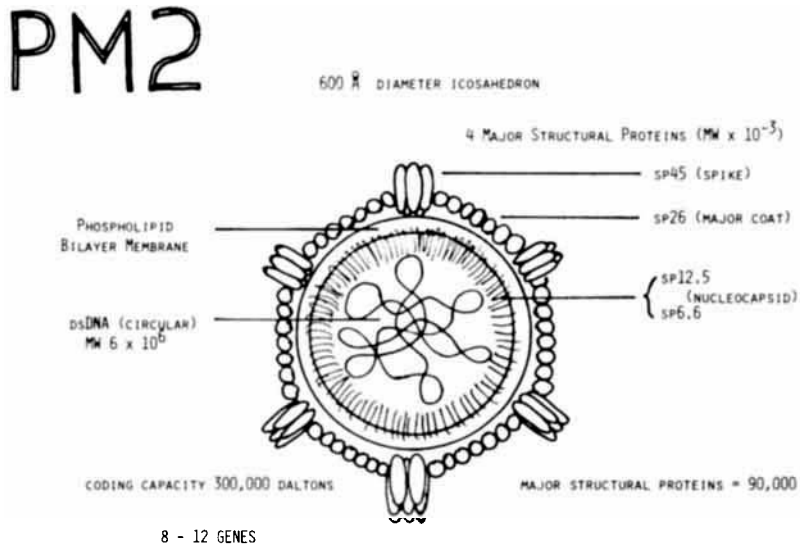

Fig. 1. Artistic rendering of cross section through icosahedral bacteriophage PM2. The double stranded closed DNA is sufficient to code for 300,000 daltons of protein or 8–12 gene products. Nucleocapsid proteins sp12.5 and sp6.6 are located between the phospholipid bilayer membrane and the DNA. Exterior to the membrane are located sp 26, the major coat protein and sp 45, the spike protein. The four major structural proteins of the virus total 90,000 daltons.

PM2 replicates in its marine host Pseudomonas Bal-31 in a typical lytic fashion (1). Figure 1 depicts the relationship of the lipid bilayer membrane to the double stranded DNA and the major coat protein as determined by x-ray diffraction (2). The disposition of these proteins in relation to the membrane has been determined by a variety of labeling techniques (3). The relative disposition of sp12.5 and sp6.6 in relation to each other, the membrane and the DNA, is uncertain beyond their association with the interior of the membrane (3) and possible proximity to the DNA (4). The size and shape of the viral membrane could be determined by 1) formation around a nucleocapsid core, 2) formation against an outer scaffolding or cage, or 3) the insertion of wedge-shaped protein into the bilayer thus causing a planar membrane to close on itself. Many membrane-containing animal viruses acquire their membrane by budding outward from the host plasma membrane. In the best studied cases of influenza and parainfluenza (5), mechanism 1 above appears to be employed. Bacteriophage PM2 cannot bud outward from the plasma membrane because of the cell wall, but rather appears to form at the inner face of the cytoplasmic membrane. Using mutants of PM2, the work described here suggests that the membrane of PM2 is not made by either mechanism 1 or 2; membrane morphogenesis does not require a nucleocapsid or an outer shell of capsid protein.

MATERIALS AND METHODS

The host of PM2, Pseudomonas Bal-31, was the gift of Dr. Wallace Snipes (Pennsylvania State University). Liquid cultures were grown in MTG (1) with forced aeration at 29° unless otherwise indicated. Virus was originally obtained from Dr. Romilio Espejo. When stored in VSM at 4° as the resuspended pellet from a lysate, the half-life of infectivity was about two months. Viral dilutions for plating were made in VSM. Plaque assays were performed on agar plates made with Q-medium (6). The isolation of temperature sensitive mutants of PM2 will be described in detail elsewhere (Brewer, in preparation).

SDS-PAGE

Pelleted material was dissolved in gel sample buffer containing 2% SDS, 62.5 mM Tris-Cl (pH 6.8), 10% glycerol, 0.002% Bromphenol Blue tracking dye and 5% mercaptoethanol, heated for 1 min at 100°, cooled and applied to a 1 mm thick stacking gel atop a resolving gel varying linearly from 17.5–12.5% in acrylamide in buffers as described (7). Electrophoresis was performed at 2.5 watts constant power (100 volts initially) for 3 hr. Gels were fixed 10 min in 50% TCA; stained 10 min in 0.1% Coomassie Brilliant Blue, 50% TCA and destained overnight in 10% acetic acid, 10% methanol. After 4 hr in 10% methanol the gel was dried by vacuum onto filter paper. Autoradiography was performed with Kodak RP/R-54 film.

Electron Microscopy of Infected Cells

Ten ml cultures of bacteria growing in MTG + CAA were infected with virus (moi = 10). After bubbling for the time indicated, cells and virus were fixed in situ by the addition of glutaraldehyde to 0.5%. After mixing, bubbling was suspended and the culture was left to fix for 30 min. Cells were then gently pelleted (3000 × g, 5 min) and let sit another 30 min without removing the supernatant. The fixed cell pellet was gently washed with 2 ml Michaelis buffer (8). Osmium fixation, dehydration, embedding in Epon and staining of ultrathin sections with uranyl acetate and lead citrate have been described (9). Sections were observed at 80 kv in a Phillips EM300, electron microscope.

RESULTS AND DISCUSSION

In order to study the control of membrane biogenesis in bacteriophage PM2, a number of conditional-lethal mutants of the virus were isolated (Brewer, manuscript in preparation), some of which may be defective in the formation of the membrane. For comparison, the appearance of an uninfected bacterium and that of cells infected by wild-type PM2 is shown in Fig. 2. Note the electron dense "filled" appearance of 50–60 nm diameter virions at the inner face of the cytoplasmic membrane.

At the restrictive temperature, cells infected with viral mutant ts 1 show a level of incorporation of ^{3}H-thymidine into TCA precipitable material midway between that of cells infected by wild-type virus and uninfected cells (Brewer, in preparation); synthesis of DNA, its processing or its packaging into the virion may be inhibited. Figure 3 shows the appearance of cells infected with ts 1 at the restrictive temperature. In both unlysed cells (lower) and partially lysed cells (upper) an abundance of "empty" virus-size membranes are seen inside the cell. These forms are not observed in cells infected with wild-type virus. At the permissive temperature in cells infected with ts 1, these vesicular forms are greatly outnumbered by normal-appearing "filled" virions. These results suggest that the synthesis of viral membrane of the correct size and shape does not require a DNA core around which it must form.

Figure 4 shows the appearance of a cell infected at the restrictive temperature with ts 5. In addition to the excess of "empty" virus-size membrane vesicles similar to ts 1, many of the membranes appear "filled." At the permissive temperature, thin sections of cells infected with ts 5 are not distinguishable from their counterparts at restrictive temperature. At the restrictive temperature, DNA synthesis in cells infected with ts 5 is equal to that of cells infected with wild-type virus.

The proteins of the virus and those of the host that are being synthesized during infection can be labeled with ^{35}S-methionine. Subsequent electrophoretic separation of the

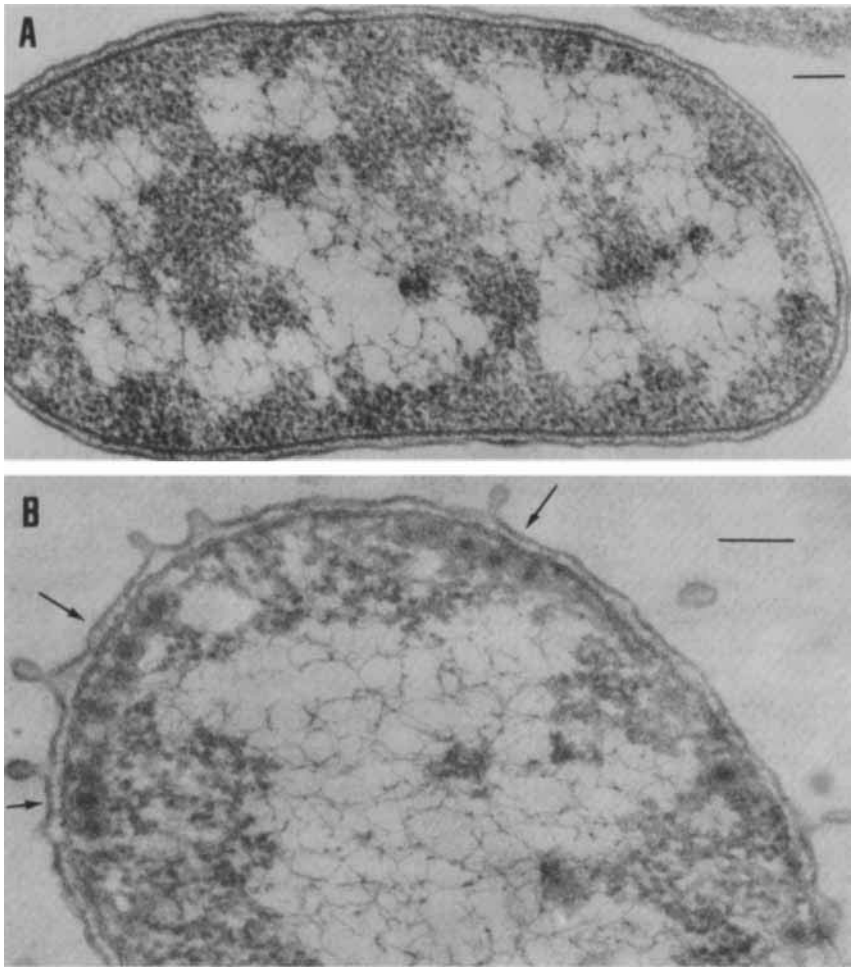

Fig. 2. Electron micrograph of a thin section of an uninfected cell (A) and a cell 45 min post-infection with wild-type virus at 30°(B). Arrows point to virion maturation at inner face of cytoplasmic membrane. Bar = 100 nm.

labeled products on the basis of size in a polyacrylamide slab gel in the presence of SDS (SDS-PAGE) reveals the four major viral structural proteins among the proteins of the host bacterium (Fig. 5, WT). In a mutant viral gene product that does not fold properly, which is likely for a temperature-sensitive mutant at the restrictive temperature, there are several possible mechanisms that would exhibit an abnormal protein pattern on SDS-PAGE. There could be a temperature-sensitive defect in the cleavage of a polypeptide precursor to produce two or more smaller polypeptides. No examples of this mechanism are yet known. Another possibility is that a structural protein of the virus does not bind properly to its neighbors in an aberrant conformation at the restrictive temperature. In cells infected with wild-type virus, the major viral capsid protein (sp26) which is located just outside the viral membrane, normally sediments with host and virus membranes. This protein made by mutant ts 5 does not pellet with those membranes (Fig. 5); instead, large amounts of capsid protein are recovered from the supernatant by acid precipitation (not shown). Thus, in cells infected with ts 5 at the restrictive temperature, sp26 may be defective in

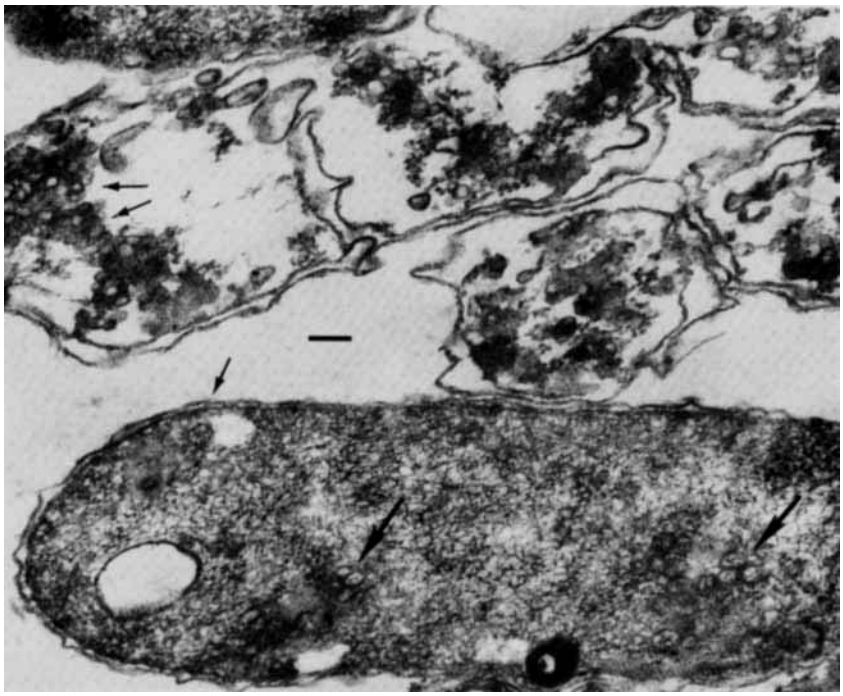

Fig. 3. Thin section of a partially lysed (upper) and an unlysed (lower) cell infected with ts 1, 90 min at 30°. Arrows point to characteristic membrane vesicles. Bar = 100 nm.

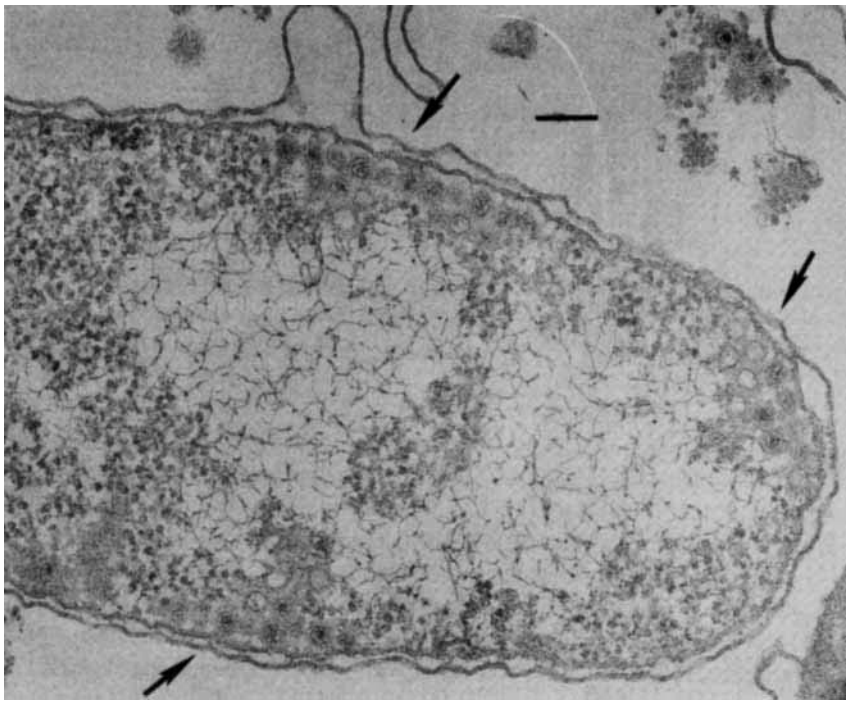

Fig. 4. Thin section of a cell infected with ts 5, 90 min at 30°. Arrows point to characteristic membrane vesicles. Bar = 100 nm.

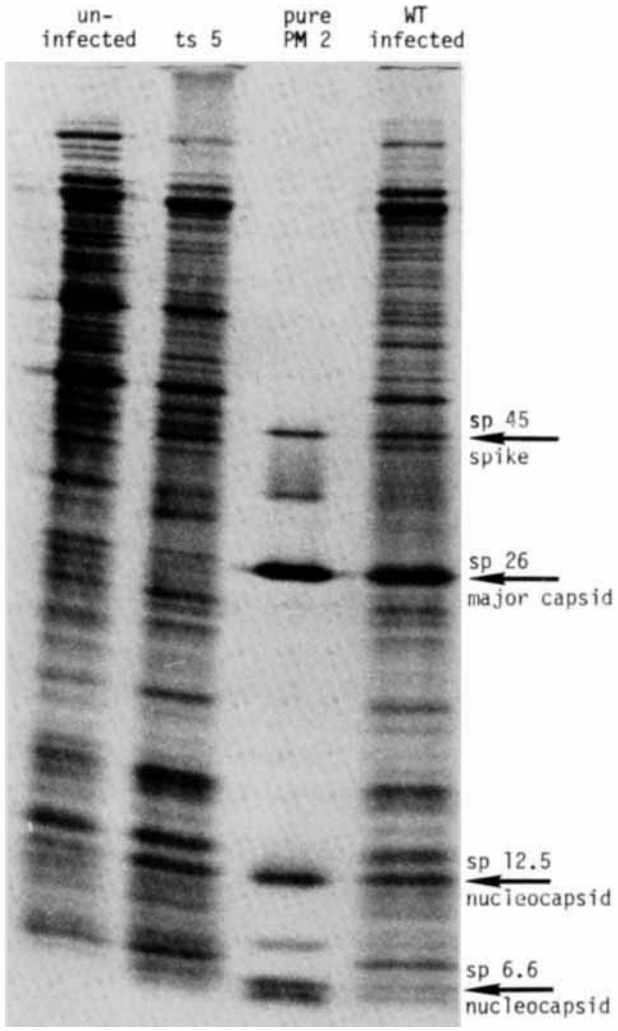

Fig. 5. SDS-PAGE autoradiogram of cells infected as indicated and labeled continuously with ^{35}S-methionine (2 μCi/ml, 1 μM). After incubation (90 min at 30°) a membrane sediment was obtained by centrifugation (150,000 × g, 45 min).

binding to the viral membrane. It appears, therefore, that viral membrane synthesis does not require the scaffolding or support of an outer shell of capsid protein sp26 in order to form a bilayer of the proper size.

Work is in progress to isolate the defective sp26 for biochemical characterization as well as the incomplete viral membrane vesicles made by ts 1 and ts 5. The characterization of these and other mutants of PM2 may indeed show that the insertion of either sp12.5 or sp6.6 into the host membrane causes a membrane of the proper size and shape to bud off into the cytoplasm. In general, the control of membrane size and shape at the microscopic level may be regulated by the control of the partitioning of hydrophobic proteins into and out of areas undergoing morphogenesis.

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