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Procoat, the Precursor of M13 Coat Protein, Inserts Post-Translationally into the Membrane of Cells Infected by Wild-Type Virus

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In growing cells infected by wild-type coliphage M13, the synthesis of procoat protein is completed before it inserts into the plasma membrane and is converted to coat protein.

The synthesis and membrane insertion of coliphage M13 coat protein (gene 8 product) has been studied (2-6, 12-15) to answer questions of membrane assembly (8, 14). Coat protein is made as a precursor, termed procoat, with 23 additional NH_2-terminal residues (10). Procoat is only synthesized by polysomes which are not attached to the plasma membrane (5). In earlier studies, cells infected by M13 or by M13 amber 7 mutant virus were pulse-labeled with [^{3}H]proline, chased with nonradioactive proline, fractionated, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography (2-5). The amber 7 mutant was used because the conversion of procoat to coat protein was slower in cells infected by this virus than in cells infected by wild-type M13 (5). In amber 7 M13-infected cells, pulse-labeled procoat is found in the cytoplasm. Procoat then binds to the inner surface of the plasma membrane and, in the presence of the transmembrane electrochemical potential, inserts into the bilayer; it is then cleaved to coat protein plus leader peptide. However, amber 7-infected cells accumulate intracellular membranes and are not viable (9), although they continue to metabolize. This has raised the question of whether coat protein biosynthesis occurs by the same pathway in these cells as in cells infected by wild-type virus.

Soluble procoat and procoat bound to the inner surface of the plasma membrane have also been detected in pulse-labeled cells infected by wild-type M13 (2-5) at 37°C. However, most of the labeled procoat was already converted to coat protein during the time of these pulses. Thus, the internal procoat seen in these experiments was not proven to be the precursor of coat protein.

We now report the kinetics of procoat synthesis, membrane insertion, and proteolytic processing in cells infected by wild-type M13 at 42°C, where procoat is metabolized more slowly that at 37°C. A clear precursor-product relationship is found between procoat and coat proteins. Procoat is only found inside the cell, hidden from

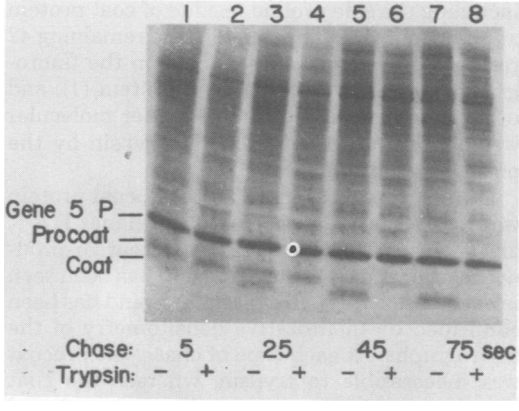

FIG. 1. *E. coli HJM114 (13) was grown at 42°C in medium containing M9 salts (7), glucose, and vitamin B_1 to an absorbance at 600 nm of 0.4 and infected with wild-type M13 at a multiplicity of 100. After 1 h, 3.2 ml of culture was pulse-labeled with [^{3}H]proline (100 μCi, 110 Ci/mmol, New England Nuclear Corp.) for 15 s and chased with 1.6 mg of proline. Portions (0.8 ml) were chilled with crushed ice, collected by centrifugation (10 min, 4°C, 30,000 × g), and suspended in 100 μl of a solution of 10% (wt/vol) sucrose, 30 mM Tris-chloride (pH 8.1), 10 mM EDTA, and 1 mg of lysozyme per ml. After 30 min at 0°C, one-half of each fraction was mixed with trypsin (0.23 mg in 100 μl of 20% sucrose-30 mM Tris-chloride [pH 8.1]-10 mM EDTA) and incubated for 30 min at 0°C. After addition of soybean trypsin inhibitor (0.4 mg, Worthington Diagnostics Corp.), proteolyzed spheroplasts were centrifuged (0°C, 15,000 × g, 10 min) mixed with 500 μl of 5% trichloroacetic acid, and prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (3). Spheroplasts which were not treated with trypsin were precipitated with 500 μl of 5% trichloroacetic acid. Lanes 1, 3, 5, and 7 were without no trypsin treatment; lanes 2, 4, 6, and 8 were with trypsin treatment. Chase times were as follows: 5 s, lanes 1 and 2; 25 s, lanes 3 and 4; 45 s, lanes 5 and 6; 75 s, lanes 7 and 8. P, Protein.*

external protease by the plasma membrane. It is post-translationally inserted into the membrane and converted to coat protein.

Escherichia coli HJM114 was grown at 42°C in minimal medium, infected by M13, and pulse-labeled for 15 s with [^{3}H]proline. Unlabeled proline was added at the end of the pulse period. After 5, 25, 45, and 75 s of chase, portions of the culture were rapidly cooled by addition to ice. Each portion was converted to spheroplasts at 0°C, and one-half of each spheroplast preparation was digested with trypsin. Each sample was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography (Fig. 1). After only 5 s of chase (lane 1), very little of the procoat was converted to the coat protein. The NH_2 terminus of the coat protein is exposed on the outer surface of the membrane (12, 13). Trypsin cleaves off the first 8 residues (13), including the sole proline residue of coat protein at position 6 (11). This renders the remaining 42 residues of coat protein invisible on the fluorograph (lane 2). Procoat, gene 5 protein (1), and other cytoplasmic proteins of higher molecular weights are protected from the trypsin by the plasma membrane (2).

During the succeeding 60-s chase, coat protein was formed as the procoat was lost (lanes 1, 3, 5, and 7). This strongly suggests a precursor-product relationship. This relationship has been seen in each of several such experiments and has been confirmed by quantitative densitometry of the fluorographs. At each time of chase, the procoat was inaccessible to trypsin, whereas the coat protein was trypsin sensitive (lanes 2, 4, 6, and 8). Although coat protein inside the cell or transmembrane procoat would be expected to be an assembly intermediate if cleavage occurred before or after insertion, respectively, neither species was abundant enough to be detected in these experiments.

Although these experiments were qualitatively similar to those reported for amber 7 M13-infected cells, *E. coli* was not killed by wild-type virus infection. Cells infected at 42°C continued to grow (Fig. 2) and shed virus. Although the rate of virus production was much lower at 42°C than at 37°C, a cell culture infected by M13 and grown overnight at 42°C showed a 10^4-fold increase in virus titer.

This post-translational assembly pathway has been reproduced in an in vitro protein synthesis incubation (J. M. Goodman, C. Watts, and W. Wickner, manuscript in preparation) with polysomes from M13-infected cells and inverted cytoplasmic membrane vesicles from *E. coli*. The availability of pure procoat (P. Silver and W. Wickner, manuscript in preparation) and leader peptidase (C. Zwizinski and W. Wickner, J. Biol. Chem., in press) may allow these reactions to be studied with defined components.

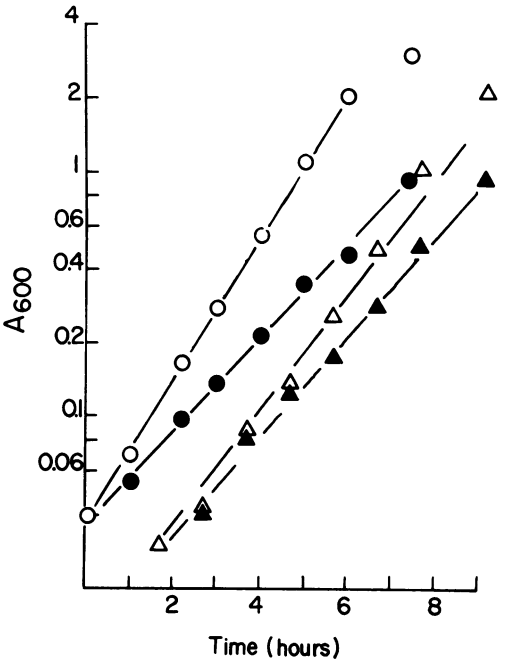

FIG. 2. *Growth of E. coli JHM114 in medium containing M9 salts, 1% glucose, and vitamin B_1 at 37 and 42°C was monitored by the absorbance at 600 nm (A_{600}). Where indicated, cells were infected by wild-type M13 at a multiplicity of 10. Symbols: ○, uninfected cells at 37°C; ●, infected cells at 37°C; △, uninfected cells at 42°C; ▲, infected cells at 42°C.*

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