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M13 procoat and a pre-immunoglobulin share processing specificity but use different membrane receptor mechanisms

(dog pancreas microsomes/bacterial leader peptidase)

COLIN WATTS, WILLIAM WICKNER, AND RICHARD ZIMMERMANN

Molecular Biology Institute and Department of Biological Chemistry, University of California, Los Angeles, California 90024

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ABSTRACT **Bacteriophage M13 procoat is accurately processed to transmembrane coat protein by salt-washed or *N*-ethylmaleimide-treated rough microsomes from dog pancreas. These treatments inhibit the processing of eukaryotic secreted protein precursors. M13 procoat can assemble into dog pancreas microsomes post-translationally. Thus, the microsomal proteins needed for assembly may be determined by the nature of the precursor protein itself. These results, and our finding that the mouse IgG κ chain fragment precursor is processed by *Escherichia coli* leader peptidase, also suggest that the cleavage specificity of leader (signal) peptidases and the properties of preproteins that render them suitable for cleavage have been conserved during evolution.**

In vivo studies have shown that assembly and processing of precursor forms is not coupled to translation (1–4) in *Escherichia coli*. A number of features, generally relevant to the assembly of *E. coli* membrane and secreted (periplasmic) proteins, have been revealed by studies of phage M13 coat protein. This is an integral component of the inner membrane during infection and is made initially as a precursor, termed procoat, with 23 extra amino acids at its amino terminus. We have found that (*i*) procoat assembles into membranes post-translationally *in vivo* (4, 5) and *in vitro* (6, 7); (*ii*) proteolytic cleavage of procoat is catalyzed by leader peptidase; (*iii*) procoat assembly *in vivo* depends on the membrane electrochemical potential (8), which is also required for the export of periplasmic and outer membrane proteins (9, 10); and (*iv*) purified leader peptidase, reconstituted into liposomes, catalyzes the cleavage and insertion of radiochemically pure procoat (7). Thus, no additional proteins appear to be required for this assembly reaction, although it is possible that other factors might influence its efficiency.

In contrast, assembly and processing of premembrane proteins by microsomal membranes from dog pancreas requires an association of the nascent chain with the membrane during translation (11, 12). Treatment of rough microsomes with buffers of high ionic strength [with (13) or without (14, 15) prior protease treatment] removes proteins required for precursor processing *in vitro*. Processing activity is restored when the extracted proteins are added back to the inactivated microsomes. Two different extracted components have now been characterized (16, 17) and both can be inactivated by treatment with *N*-ethylmaleimide (18). "Signal recognition particle" is released from microsomes by high salt treatment. It will bind specifically to polysomes with nascent chains of preprolactin and block further polypeptide elongation. Addition of microsomes restores the translation of preprolactin and results in its processing to prolactin and sequestration (16). Meyer *et al.* (17) have isolated a microsomal protein of 72,000 daltons. A fragment of this protein is removed from the microsomes by proteolysis and high salt. Microsomes thus inactivated were reactivated upon readdition of the fragment (13). This protein, called "docking protein" (19), interacts with signal recognition particle to relieve the inhibition of polypeptide elongation (19). These proteins provide a mechanism for directing the specific binding of nascent proteins to rough microsomes.

We have asked how M13 procoat, which assembles post-translationally *in vivo* and *in vitro*, would interact with the microsomal system. Three possible results were anticipated: (*i*) failure of the eukaryotic processing system to recognize the prokaryotic precursor; (*ii*) processing and insertion of procoat, which depends on the salt-extractable- or *N*-ethylmaleimide-sensitive components; or (*iii*) processing and insertion occurring independently of the extractable components. We show here that the last result is obtained. We also present data showing that the cleavage specificity of leader peptidase has been conserved in evolution.

MATERIALS AND METHODS

Rabbit anti-mouse κ chain serum and a wheat germ lysate were from Miles and Bethesda Research Laboratories, respectively. Proteinase K was from Sigma (type XI). Reticulocyte lysate was from New England Nuclear.

RNA Isolation. RNA was isolated from M13-infected *E. coli* (strain HJM 114) by the method of LaFarina and Model (20) and translated in the cell-free system previously described (21). Randolph Wall of this institute kindly provided the mouse cell line 45.6 (clone E1), originally derived from the plasmacytoma line MPC-11 (22). Total cytoplasmic RNA was prepared from these cells by phenol/chloroform phase partition.

***In Vitro* Translation and Immunoprecipitation.** Mouse cell RNA (7 A_{260} units/ml) was translated in a wheat germ extract for 60–90 min at 25°C. For immunoprecipitation, samples were analyzed by either of two methods. In method A, samples were mixed with 4–5 vol of 2% Triton X-100/0.15 M NaCl/10 mM Tris·HCl, pH 8.0/1 mM EDTA and centrifuged in the Beckman Airfuge for 15 min. The supernatants were mixed with 0.5 ml of the same buffer at 4°C. Alternatively, in method B, 0.25 vol of 10% $NaDodSO_4$ was added and the samples were boiled for 3 min; then 20 vol of Triton buffer was added. Rabbit anti-mouse κ chain serum (5 μl) was added, followed by addition of 20 μl of a 10% suspension of killed, formalin-fixed *Staphylococcus aureus* and the mixture was incubated at 4°C for 1 hr with agitation. The bacteria and adsorbed immunoglobulins were washed twice with 0.5 ml of the Triton buffer and once in the same buffer without Triton. Immunoprecipitation of procoat

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Abbreviation: $C_κ$, constant region fragment of a κ light chain.

and coat protein with an affinity-purified antibody (23) followed the same protocols. $NaDodSO_4$ gel electrophoresis and fluorography were performed as described (4).

Preparation of Microsomes.* Dog pancreas microsomes were prepared from fresh tissue (24, 25). All buffers contained 0.1% 2-mercaptoethanol and 40 μg of phenylmethylsulfonyl fluoride per ml. The pellet of rough microsomes was resuspended in 0.25 M sucrose/20 mM Hepes, pH 7.5/50 mM KCl/1 mM dithiothreitol. Nuclease treatment was performed as described (26) and was followed by the addition of 5 mM EDTA to dissociate the ribosomes. The microsomes were collected by centrifugation through 0.5 M sucrose/20 mM Hepes, pH 7.5/50 mM KCl/2 mM magnesium acetate/1 mM dithiothreitol, resuspended in the same buffer, but containing 0.2 M sucrose, and stored at −70°C.

RESULTS

Assembly of M13 Procoat into Microsomes. The inclusion of rough microsomes during the cell-free synthesis of M13 procoat generated an additional product which comigrated with M13 coat protein (Fig. 1). The orientation of procoat and coat protein was assayed by proteolytic digestion. Procoat synthesized in the presence of microsomes was sensitive to the added protease, whereas the coat protein was resistant but displayed a slightly increased mobility. This species, termed coat' (coat prime), is derived from transmembrane coat protein oriented with the NH_2-terminal domain protected within the vesicle lumen (6, 27). Coat' was immunoprecipitable with an antibody specific for the NH_2-terminal octapeptide of coat protein (23), confirming this orientation (Fig. 1).

To determine if the same cleavage was made by the eukaryotic and prokaryotic processing systems, [^{3}H]proline was included as the labeled amino acid in the translation system and the putative coat protein was isolated by electrophoresis. Proline emerged at the 6th cycle in the automated Edman degradation (Fig. 2). When [^{3}H]phenylalanine was the labeled amino acid, radioactivity emerged at the 11th cycle (Fig. 2). These are the expected positions for these amino acids in authentic coat protein (28). These data show clearly that procoat is cleaved at the same position by both the microsomal leader peptidase and by purified leader peptidase from *E. coli* (29).

In view of the evidence that components in addition to leader peptidase are involved in microsomal processing of eukaryotic precursors, we prepared microsomes that had been modified by treatment with *N*-ethylmaleimide (18) or with buffers of high ionic strength (15). Microsomes that had been treated with *N*-ethylmaleimide were included in reactions synthesizing M13 procoat (Fig. 3*A*, lanes 2–5). The same amounts of mock-treated microsomes were tested in control reactions (Fig. 3, lanes 6–9). *N*-Ethylmaleimide treatment had no effect on the processing of procoat to coat protein. Moreover, the coat protein generated by the *N*-ethylmaleimide-treated microsomes was integrated into the membrane, as judged by its resistance to digestion by proteinase K in the absence of detergent, but not in its presence (Fig. 3*A*, lanes 10–12). Processing of procoat to coat protein was also insensitive to treatment of the microsomes with buffers of high ionic strength (Fig. 4*A*).

To confirm that these treatments had indeed modified the microsomal membrane, we tested the treated membranes for their ability to process the precursor of an IgG light chain fragment, C_κ. This protein is synthesized by the mouse plasmacytoma MPC-11 (30) and is the product of an aberrantly rearranged κ gene (31). This κ fragment is made as a precursor of

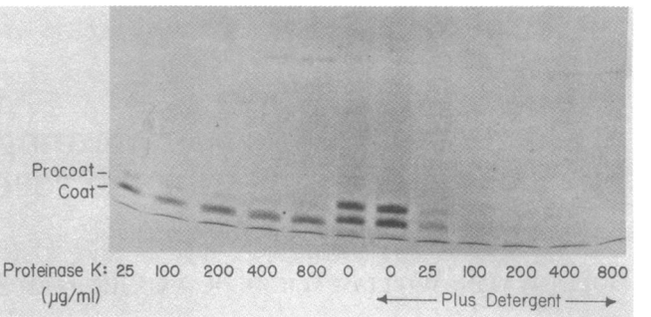

FIG. 1. Processing and sequestration of M13 coat protein by dog pancreas microsomes. M13 RNA was translated in a bacterial extract in the presence of [^{35}S]methionine and dog pancreas microsomes (1.75 A_{280} units) at 37°C for 60 min. Aliquots were incubated at 4°C for 60 min with 0.8 M sucrose and proteinase K at the concentrations shown, in the presence or absence of 0.5% Triton X-100. Proteolysis was stopped by the addition of phenylmethylsulfonyl fluoride (final concentration, 2 mM) and $NaDodSO_4$ (final concentration, 2%). The samples were analyzed by immunoprecipitation (method B, *Materials and Methods*), followed by $NaDodSO_4$ gel electrophoresis and fluorography.

M_r 11,500, pre-C_κ (30). When RNA isolated from the MPC-11 line (clone 45.6 E1) was translated in a wheat germ lysate in the presence of microsomes, the pre-κ fragment was proteolytically processed (Fig. 5, lane 2) and sequestered within the microsomal vesicles. Sequestration was shown by the resistance of the mature form of the light chain fragment to proteolytic digestion (Fig. 5, lane 3), which was lost in the presence of detergent (Fig. 5, lane 4). *N*-Ethylmaleimide treatment of the microsomes inhibited their ability to process the precursor of

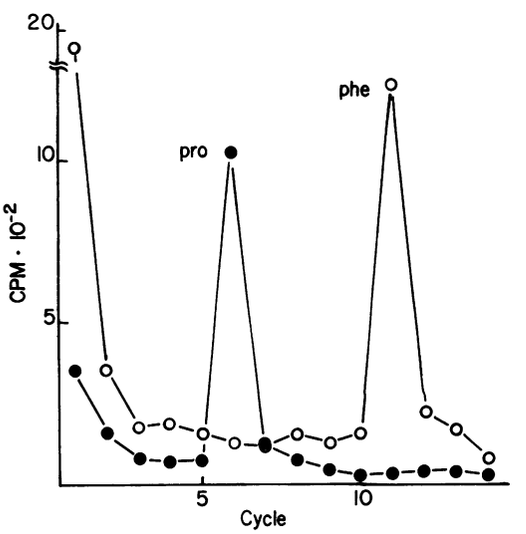

FIG. 2. Dog pancreas microsomes process procoat to authentic coat protein. M13 mRNA was translated in the presence of [^{3}H]proline or [^{3}H]phenylalanine and dog pancreas microsomes (2–5 A_{280} units). After 60 min at 37°C, the reactions were boiled in $NaDodSO_4$ and the coat protein was collected by immunoprecipitation (method B). The immunoprecipitates adsorbed to *S. aureus* were washed (see *Materials and Methods*) and the [^{3}H]phenylalanine-labeled protein was dissociated from the bacteria by boiling in 1% $NaDodSO_4$ and was analyzed by automated Edman degradation. The [^{3}H]proline-labeled protein was separated by $NaDodSO_4$ gel electrophoresis and fluorography. The band comigrating with coat protein was cut out of the dried gel, rehydrated in 1 ml of H_2O, and washed in a further 1 ml of H_2O. The putative coat protein was eluted by boiling in two successive 0.4-ml aliquots of 1% $NaDodSO_4$. Approximately 1 mg of preimmune rabbit IgG was added as a carrier and the sample was analyzed by Edman degradation. The derivatized amino acids obtained at each cycle were solubilized in ethyl acetate and assayed for tritium. pro, Proline; phe, phenylalanine.

* The A_{280} of microsomes in complex *in vitro* translation mixtures is the absorbance that would be due to the microsomes alone.

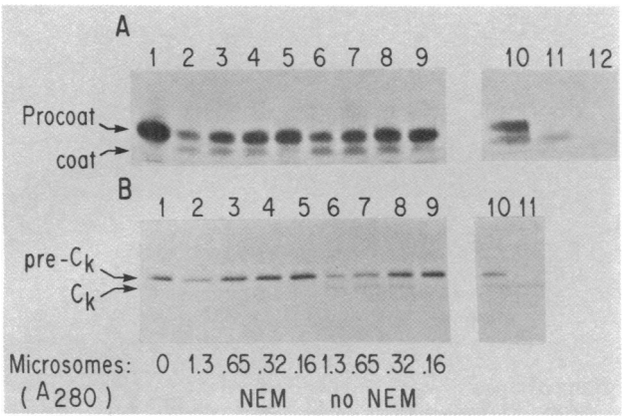

FIG. 3. Effect of *N*-ethylmaleimide treatment on precursor processing by dog pancreas microsomes. Dog pancreas microsomes (28 A_{280} units) were treated with 1 mM *N*-ethylmaleimide for 30 min at 25°C as described by Jackson *et al.* (18) but without Trasylol. The microsomes were sedimented (5 min, Beckman Airfuge) to the interface between 10 mM triethanolamine·HCl, pH 7.5/1 mM dithiothreitol/0.5 M sucrose and a cushion of the same buffer containing 2.0 M sucrose. Another sample of microsomes was carried through in parallel but without addition of *N*-ethylmaleimide (mock-treated). The microsomes were included, at the levels indicated, in *in vitro* reactions synthesizing M13 procoat (*A*, bacterial extract) or mouse κ chain fragment (*B*, wheat germ extract). Mock-treated, lanes 6–9; *N*-ethylmaleimide-treated, lanes 2–5. To demonstrate correct assembly, *N*-ethylmaleimide-treated microsomes (2.6 A_{280} units) from a reaction synthesizing M13 procoat were subjected to post-translational proteolysis (1 hr, 0°C). (*A*) Lane 10, no protease; lane 11, 300 μg of proteinase K per ml; lane 12, 300 μg of proteinase K per ml with 1% Triton X-100. Samples were processed as described in the legend to Fig. 1. As controls for the experiments in *B*, *N*-ethylmaleimide-treated microsomes and mock-treated microsomes (0.6 A_{280} unit of each) were included in a reaction synthesizing the κ chain fragment (lane 10). *N*-Ethylmaleimide-treated microsomes (0.6 A_{280} unit/ml) were included in a κ chain fragment synthesis reaction in the presence of 0.5% Trition X-100 (lane 11). Immunoprecipitation of the constant region fragment of a κ light chain, C_κ, and pre-C_κ was by method A (see *Materials and Methods*). NEM, *N*-ethylmaleimide.

the light chain fragment (Fig. 3*B*, compare lanes 2–5 and 6–9). Mixing *N*-ethylmaleimide-treated and mock-treated membranes gave the same level of processing that was observed when mock-treated membranes alone were used, showing that inhibition was not due to residual *N*-ethylmaleimide (Fig. 3*B*, lane 10). Conditions for post-translational processing of the light chain fragment by microsomal detergent extracts were established (see Fig. 7) and were used to confirm that treatment with 1 mM *N*-ethylmaleimide does not inactivate the microsomal peptidase (ref. 18; Fig. 3*B*, lane 11).

Salt-washed membranes were also relatively inactive in processing the κ chain fragment precursor (Fig. 4*B*, lanes 4 and 5). Although the absolute amount of mature κ fragment generated by the untreated membranes was only 3- to 4-fold greater than that generated by the salt-washed membranes (Fig. 4*B*, compare lanes 2 and 4), protein synthesis was inhibited ≈75% by the untreated membranes. Quantitative densitometry of lanes 2 and 4 in Fig. 4*B* showed that only 5% of the κ fragment was processed by the treated membranes, whereas 43% was processed by the same amount of the untreated membranes.

It remained possible that the bacterial extracts that synthesize procoat also supply an ingredient which replaces the *N*-ethylmaleimide-sensitive components from microsomes. Therefore, we synthesized procoat in a reticulocyte extract (Fig. 6*A*, lane 1) in which the processing of the pre-κ fragment is sensitive to *N*-ethylmaleimide treatment of the microsomes (Fig. 6*B*). Comparable processing of procoat to coat was seen with mock-treated microsomes (Fig. 6*A*, lanes 2 and 3) or *N*-ethylmaleimide-treated microsomes (Fig. 6*A*, lanes 4 and 5).

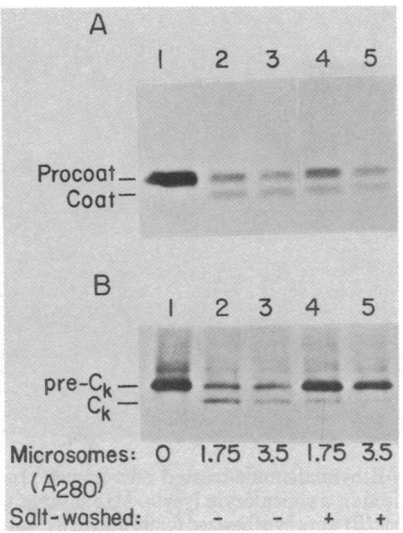

FIG. 4. Effects of salt-washing on processing of M13 procoat and mouse κ chain fragment by dog pancreas microsomes. Salt-washed microsomes were prepared by the method of Walter and Blobel (15) with the following modifications. One hundred microliters of microsomes (40 A_{280} units) in 0.2 M sucrose/20 mM Hepes, pH 7.5/50 mM KCl/2 mM magnesium acetate/1 mM dithiothreitol was mixed with 50 μl of 1.5 M potassium acetate/15 mM magnesium acetate (pH 7.5) and incubated at 0°C for 15 min. The microsomes were reisolated by centrifugation in the Beckman Airfuge by using a step gradient of 2 M and 0.5 M sucrose in 0.5 M potassium acetate/5 mM magnesium acetate/1 mM dithiothreitol/50 mM triethanolamine·HCl, pH 7.5. The microsomes were collected from the interface and adjusted to contain 0.25 M sucrose in the same buffer. After *in vitro* synthesis as in Fig. 3 *A* and *B*, samples were analyzed by immunoprecipitation (method B) and $NaDodSO_4$ gel electrophoresis.

These results show that procoat does not need to recruit the *N*-ethylmaleimide-sensitive and salt-extractable components for its integration and processing by microsomes. Because there is a mechanistic connection between salt-extractable proteins and a cotranslational assembly reaction, we tested whether the procoat processing reaction would proceed post-translationally. After 1 min of procoat synthesis in the presence of [^{35}S]methionine, chloramphenicol was added to stop translation. After 30 sec, microsomes were added and incubation was continued for 30 min. Approximately equal radioactivities were recovered in procoat and coat (Fig. 7, lane 3); because procoat has three methionyl residues (32) and coat has one, this corresponds to 75% conversion. Control incubations showed that processing was occurring prior to the addition of $NaDodSO_4$ (Fig. 7, lane 2) and that the chloramphenicol was completely blocking protein synthesis (Fig. 7, lane 1).

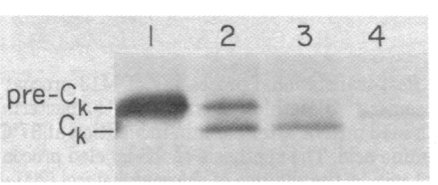

FIG. 5. Mouse IgG κ chain fragment precursor is processed and sequestered by dog pancreas microsomes. mRNA from the mouse cell line MPC-11 (clone 45.6 E1) was translated in the wheat germ extract in the absence (lane 1) or presence (lanes 2–4) of dog pancreas microsomes. Post-translational proteolysis of microsomes was performed as described in the legend to Fig. 1. Lane 2, no addition; lane 3, 300 μg of proteinase K per ml; lane 4, 300 μg of proteinase K per ml with 1.0% deoxycholate.

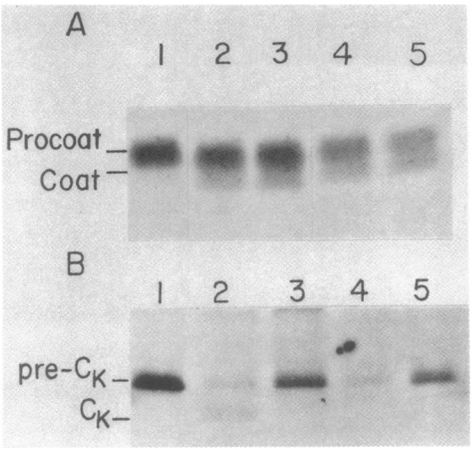

FIG. 6. *N*-Ethylmaleimide-treated microsomes process M13 procoat synthesized in a reticulocyte lysate. M13 procoat (*A*) and κ fragment precursor (*B*) were synthesized for 90 min at 37°C in a reticulocyte lysate in the presence of [^{35}S]methionine and either M13 mRNA (2 A_{260} units) or MPC-11 mRNA (7 A_{260} units). Synthesis was in the absence of membranes (lane 1, *A* and *B*) or in the presence of mock-treated microsomes (lanes 2 and 3: in *A*, 0.5 and 1 A_{280} unit, respectively; in *B*, 2 and 1 A_{280} unit, respectively) or *N*-ethylmaleimide-treated microsomes (lanes 4 and 5: in *A*, 0.5 and 1 A_{280} unit, respectively; in *B*, 2 and 1 A_{280} unit, respectively). Samples were analyzed by immunoprecipitation (method B) and $NaDodSO_4$ gel electrophoresis.

Processing by Detergent Extracts of Microsomal Membranes. We found that the light chain fragment was processed post-translationally to the mature form by extracts of microsomes made with Triton X-100 (Fig. 8*A*, lane 5), *n*-octyl glucoside (Fig. 8*A*, lane 7), or Nikkol (not shown). Processing was not observed with deoxycholate-solubilized membranes (not shown). Indeed, the presence of this detergent inhibited cleavage by the non-ionic detergent extracts (Fig. 8*A*, lane 8). Purified *E. coli* leader peptidase (33) also processed the light chain fragment precursor to its mature size (Fig. 8, lanes 2 and 3). Processing of both procoat and this eukaryotic preprotein *in vivo* occurs by cleavage between two alanine residues (30).

Despite the ability of intact microsomes to process procoat to coat (Fig. 1), procoat was not cleaved post-translationally (Fig. 8*B*, lane 3) or cotranslationally (not shown) by the same Triton X-100 extract of microsomes that cleaved the light chain fragment. *n*-Octyl glucoside (Fig. 8*B*, lane 6) and deoxycholate (Fig. 8*B*, lane 7) extracts of microsomes were also unable to cleave procoat. Both the prokaryotic and eukaryotic precursors were cleaved by *E. coli* leader peptidase in the presence of Triton X-100 (Fig. 8*A*, lanes 2 and 3 and Fig. 6*B*, lane 2). No inhibitors of the microsomal peptidase, which acts on the light chain precursor, could be found in the prokaryotic translation mixture (Fig. 8*A*, lane 6) nor could inhibitors of *E. coli* leader peptidase be demonstrated in the solubilized microsomes (Fig. 8*B*, lane 5). These results suggest that either different microsomal peptidases act on procoat and the light chain fragment or that Triton X-100, though the detergent of choice for the cleavage of procoat by *E. coli* leader peptidase, might be unsuitable for the action of the eukaryotic peptidase on procoat. The same enzyme in Triton X-100 might still act on the eukaryotic precursor.

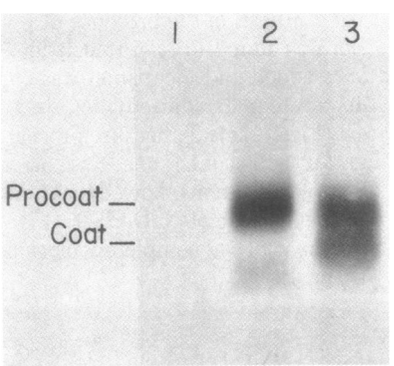

FIG. 7. Post-translational processing of M13 procoat by dog pancreas microsomes. [^{35}S]Methionine was added to a bacterial translation extract with M13 mRNA after a 5-min incubation at 37°C without radioactive amino acid. The synthesis of ^{35}S-labeled procoat was terminated after 1 min by the addition of chloramphenicol (200 μg/ml). After 30 sec, microsomes (10 A_{280} units) were added to one portion of the reaction (lane 3). After 30 min at 37°C, samples were boiled in $NaDodSO_4$ and immunoprecipitated by method B. After $NaDodSO_4$ addition, microsomes were added to the sample that had been incubated post-translationally in the absence of microsomes (lane 2) to eliminate the possibility that proteolysis of procoat occurred during the subsequent immune precipitation. In a third reaction, chloramphenicol was added prior to the [^{35}S]methionine to demonstrate the efficient termination of synthesis (lane 1).

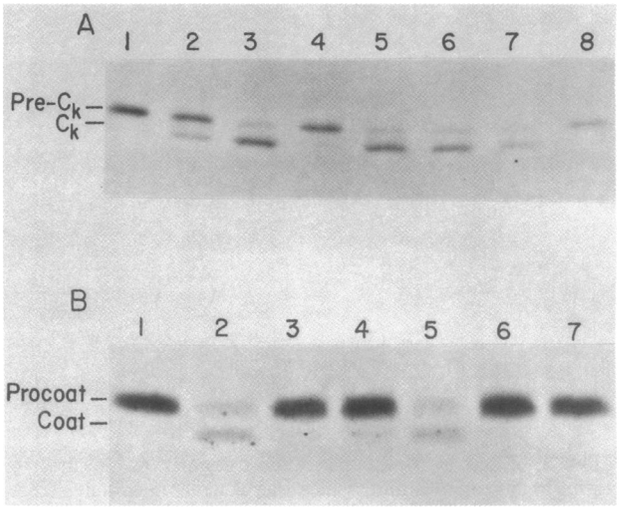

FIG. 8. Post-translational processing of mouse κ fragment and M13 procoat by microsomal extracts and *E. coli* leader peptidase. (*A*) The κ fragment precursor was synthesized in a wheat germ translation system. Portions (18 μl) were further incubated at 25°C for 60 min in the presence of: lane 1, no addition; lanes 2 and 3, 1.3 μg and 33 μg of purified *E. coli* leader peptidase, respectively; lanes 4–8, dog pancreas microsomes (2 A_{280} units). Samples in lanes 1–3, 5, 6, and 8 contained 0.5% Triton X-100; lane 7 contained 0.5% *n*-octyl glucoside; lane 8 contained 0.5% deoxycholate; lane 4 contained no detergent; lanes 5 and 6 are the same except that lane 6 contained all of the components of an M13 procoat translation reaction at the levels present in *B*. (*B*) M13 procoat was synthesized *in vitro* with an *E. coli* translation system containing 0.5% Triton. Portions (8 μl) were further incubated at 25°C for 90 min in a final volume of 40 μl. To improve procoat solubility, 5 mM EDTA was present. Lane 1, no addition; lanes 2 and 5, 4 μg of purified *E. coli* leader peptidase; lanes 3–7, dog pancreas microsomes (2 A_{280} units). Lanes 1, 2, 4, and 5 contained 0.5% Triton X-100. Lanes 6 and 7 contained 0.5% *n*-octyl glucoside and deoxycholate, respectively. Lane 3 contained no detergent. Samples were analyzed by $NaDodSO_4$ gel electrophoresis and fluorography performed after immunoprecipitation.

The cleavage of a eukaryotic precursor protein by bacterial leader peptidase shows that some common feature of precursor proteins has been remarkably conserved among species. The results also point to the critical importance of the choice of detergents and lipids in cell-free post-translational cleavage reactions.

DISCUSSION

The common features of protein secretion and membrane assembly in bacterial and eukaryotic cells are underscored by re-

cent reports that a cloned protein generated by gene fusion and including rat preproinsulin is correctly cleaved and secreted in *E. coli* (34). We have begun to investigate these comparative mechanisms by means of cell-free synthesis of bacterial and microsomal precursor proteins.

Proteins insert into (or across) a membrane in three steps: (*i*) binding to the correct membrane, (*ii*) transfer of at least part of the polypeptide chain across the lipid bilayer, and (*iii*) covalent modification, such as removal of a leader peptide. The results reported here demonstrate marked differences between bacterial procoat and eukaryotic pre-κ light chain fragment with regard to the first step, specific binding, as well as marked similarities in the last step, leader peptide removal.

Although M13 procoat assembles efficiently into dog pancreas microsomes and is cleaved accurately to coat protein (Figs. 1 and 2), it does so without using the microsomal receptor complex (signal recognition particle and docking protein), a complex which causes cotranslational insertion and which is affected by salt and *N*-ethylmaleimide (Figs. 3 and 4). This agrees well with our observations that procoat assembles post-translationally into the cell membrane *in vivo* (4, 5, 8), into isolated *E. coli* plasma membrane vesicles (6), into microsomes (see *Results*), and into liposomes where leader peptidase is the sole protein (7, 35). Presumably procoat uses a subset of the microsomal components utilized by other precursors. Our results suggest that the action of the microsomal leader (signal) peptidase(s) is not tightly coupled to the action of the *N*-ethylmaleimide- and salt-sensitive components. This is strongly supported by the finding that procoat can be post-translationally processed by microsomes (Fig. 7).

We do not expect that assembly without directing elements such as signal recognition particle is a major pathway of assembly into the rough endoplasmic reticulum *in vivo*. Whereas in bacteria all secreted (periplasmic) and membrane proteins are synthesized facing a single (inner) membrane, in eukaryotic cells a choice must be made among the various membranes. Complexes such as signal recognition particle (16) or specific receptors in the outer membrane of the mitochondrion (36, 37) may be involved in the process of choice. It will be interesting to determine whether microsomal proteins such as cytochrome b_5 and NADH cytochrome b_5 reductase, which are made on free polysomes and which lack leader sequences (38), are recognized by the microsomal receptor complex.

The results presented here do not bear on the mechanism of the second step of assembly, the transit of polypeptide across the bilayer. Procoat can spontaneously integrate into a membrane (7, 21), and it is possible that it does so with microsomes. It is not known how eukaryotic preproteins cross the microsomal membrane.

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