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Export of Protein in Bacteria†

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INTRODUCTION

One of the fundamental unanswered questions in biology is how do proteins made in one subcellular compartment reach their final destination in another. This process, which we shall call export, involves both insertion of proteins into membranes and passage of hydrophilic proteins through hydrophobic membrane barriers. In eucaryotes it is an essential step in secretion of protein and biogenesis of organelles. Although bacterial cells do not contain organelles, they do have distinct compartments and some secrete proteins. Gram-negative bacteria such as *Escherichia coli* have soluble proteins localized to a periplasmic space that is contained between the cytoplasmic and outer membranes. Thus, proteins synthesized in the cytoplasm are exported to final destinations in both the outer membrane and the periplasm. The translocation of proteins into the periplasmic space is analogous to the initial step of secretion in higher organisms, namely, the transfer of secretory proteins through the membrane into the lumen of the rough endoplasmic reticulum. Both systems involve synthesis of proteins in precursor forms that are matured by proteolytic removal of amino-terminal extensions. These extensions, usually between 15 and 30 amino acids in length (referred to as signal sequences or leader sequences), comprise a hydrophobic sequence of at least 11 amino acid residues preceded by a short stretch of residues at the amino terminus that normally includes several positive charges (for reviews, see references 14, 27, and 35, and V. A. Bankaitis, J. P. Ryan, B. A. Rasmussen, and P. J. Bassford, Jr., *in* P. Knauf and J. Cook, ed., *Membrane Protein Biosynthesis and Turnover*, in press).

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MODELS FOR EXPORT

The signal hypothesis, based on data obtained from investigations of eucaryotic secretion, postulates that a complex proteinaceous apparatus is responsible for passage of polypeptides through the membrane of the rough endoplasmic reticulum (3–5, 28). The current version of this hypothesis proposes that as soon as the signal sequence emerges from the ribosome it interacts with a signal recognition particle (Fig. 1) that exists free in the cytosol (25, 40, 42). The binding of this particle blocks further elongation (41) until the entire complex interacts with a membrane-associated docking protein, resulting in release of the block of protein synthesis (26). Then the nascent polypeptide chain is continuously extruded, as it is elongated, through a proteinaceous channel in the membrane. A processing enzyme located on the luminal side of the membrane proteolytically removes the signal.

A different model was proposed by Wickner (43, 44), originally to account for data collected in investigations with the filamentous phage M13, which inserts its coat protein into the cytoplasmic membrane of the host, *E. coli*, during infection. The coat protein was shown to be synthesized as a precursor, free in the cytoplasm, and to be inserted into the membrane only after its synthesis was complete (10, 16, 17). Subsequent insertion across the lipid bilayer and proteolytic removal of the leader sequence required the electrochemical membrane potential (8, 11). The membrane-triggered folding hypothesis (Fig. 2) rationalizes these data as follows. The role of the amino-terminal leader sequence is not to mediate binding of nascent polypeptides to the membrane, but rather to confer a conformation on the precursor that renders it soluble. Interaction of this precursor with the membrane triggers a change in conformation, resulting in association with the cytoplasmic side of the membrane. Proton motive force effects insertion across the lipid bilayer. Since the coat protein is a transmembrane protein of only 50 amino acid residues, the model was modified for application to larger, exported proteins. Wickner postulated (44) that an amino-terminal domain of a polypeptide might fold so that it could interact with the membrane while nascent, triggering a conformational change that in concert with proton motive force would result in translocation of the domain through the membrane. Thus, it is not implicit in the membrane-triggered folding hypothesis that translocation occur entirely after synthesis of the protein has been completed.

Each model specifies precise relationships in the steps of a complex phenomenon that includes synthesis of polypeptides as well as their translocation through membranes. Synthesis of protein can be described by the steps initiation, elongation, and termination of polypeptide chains. In the special case of proteins that are to be translocated through membranes, there are additional steps to consider: interaction with the membrane, transfer through the lipid bilayer, proteolytic removal of the leader sequence, and release of the matured, fully elongated protein from the membrane. Elucidation of clear temporal relationships among these steps led to proposals for mechanisms of translocation. For example, demonstration that export must be initiated during the synthesis of a protein, that is, cotranslationally, led, as a facet of the signal hypothesis, to the proposal of an obligatory, mechanistic coupling of translocation with elongation. This proposal, for which there is no direct evidence, is consistent with a large body of data and has been accepted widely even though there is now considerable evidence, obtained in bacterial systems, that contradicts it. It is important to remember that a model is valuable only insofar as it serves to guide further investigation; a model is dangerous when it is so appealing that data which are consistent are taken as proof, thereby eliminating alternative hypotheses.

Our working model (Fig. 3) describing export of periplasmic proteins in *E. coli* combines features of both the signal hypothesis and the membrane-triggered folding hypothesis and incorporates data accumulated in several labo-

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† L.L.R. wishes to dedicate this review to Helen and Lowell Randall in acknowledgment of their constant support.

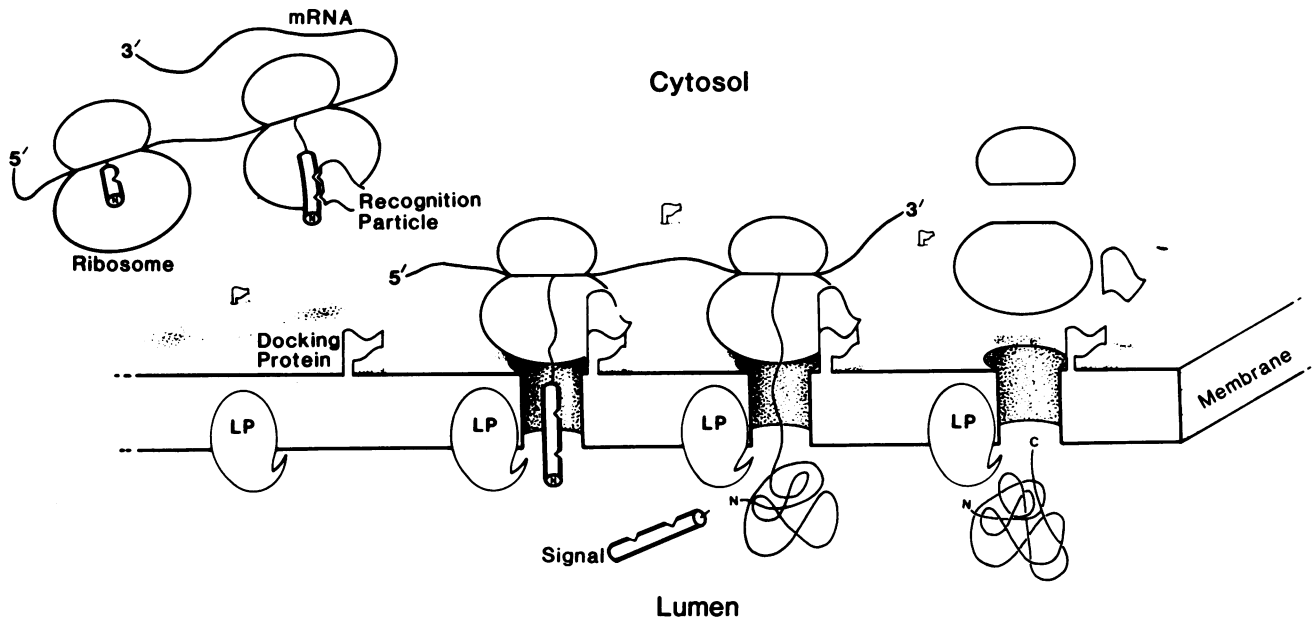

FIG. 1. Signal hypothesis: secretion of a protein through the membrane of the rough endoplasmic reticulum. See text for a detailed explanation. LP, Leader peptidase. The docking protein and leader peptidase are represented as membrane-associated proteins.

ratories. In this model, the initial recognition events are depicted as set forth in the signal hypothesis. Supporting evidence comes from genetic studies. The critical role of the signal sequence in initiating export has been repeatedly demonstrated by isolation of strains carrying mutations in the signal sequence of a variety of exported proteins (2, 12; for review, see reference 27 and Bankaitis et al., in press). These mutations prevent export and result in accumulation of the precursor forms within the cell. The characterization of extragenic suppressors of these signal sequence mutations as well as of other mutants that are pleiotropically defective in export supports the notion that the export machinery

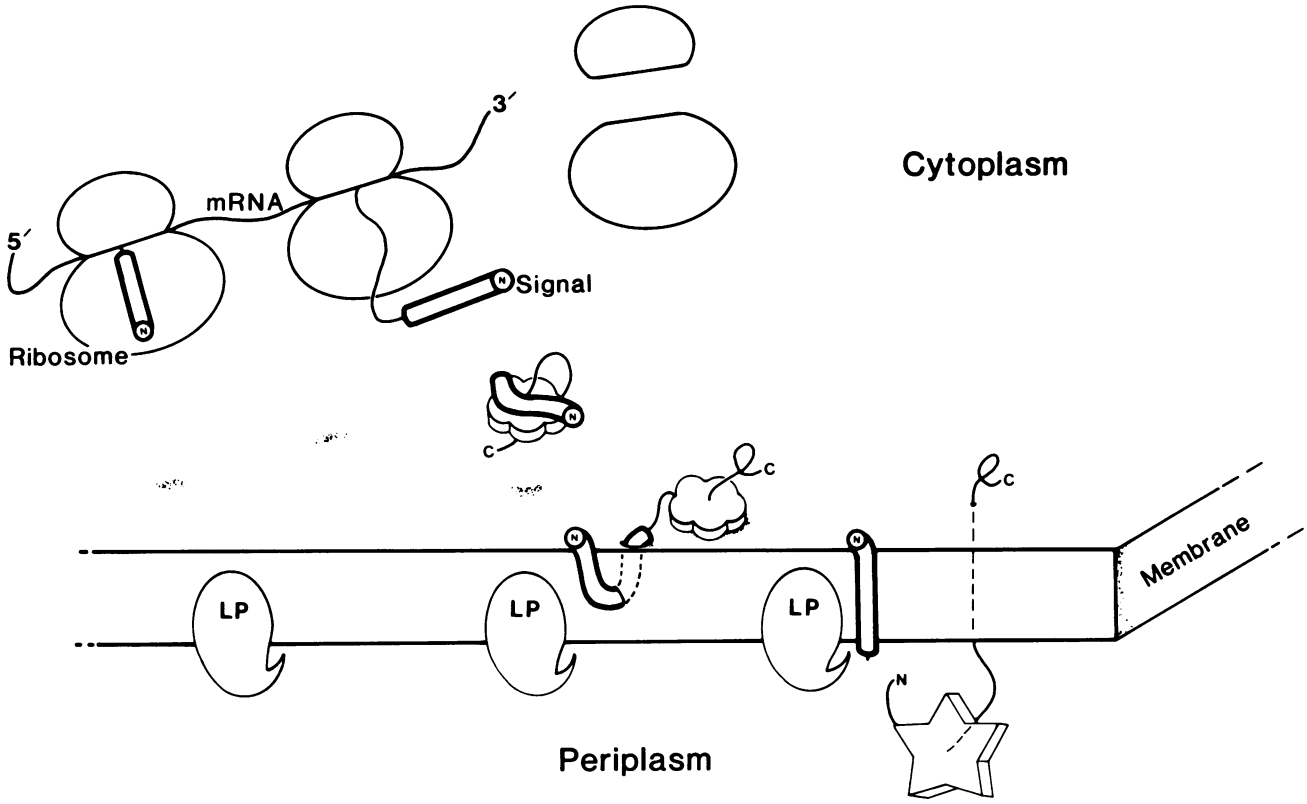

FIG. 2. Membrane-triggered folding hypothesis: insertion of the coat protein of phage M13 into the cytoplasmic membrane of *E. coli*. See text for a detailed explanation. LP, Leader peptidase. The flower and star represent unspecified conformations.

comprises several proteinaceous components. The numerous genes identified to date and the roles that their gene products might play in export are discussed at length in recent reviews (27; Bankaitis et al., in press). Here we shall make only the summary statement that the signal sequence functions in concert with several proteins, both soluble and membrane associated, to bring the polysome to the site of export on the membrane.

There is direct evidence that some exported proteins are synthesized on polysomes that are associated with membrane. Smith et al. (37) labeled nascent polypeptides of *E. coli* from outside the cell, using a reagent that does not penetrate the cytoplasmic membrane, and concluded that ribosomes carrying these incomplete polypeptides were engaged in the export process. Nascent alkaline phosphatase, a periplasmic protein, was identified among the polypeptides labeled, further supporting the conclusion. A similar study with the nascent proteins of *Bacillus subtilis* gave similar results (36). Using another approach, Randall and Hardy (31) showed that exported proteins were preferentially synthesized on membrane-bound polysomes. The set of exported proteins for which there is direct evidence for membrane-associated synthesis includes, in *E. coli*, the outer membrane protein LamB (32) and the periplasmic proteins, maltose-binding protein (31), arabinose-binding protein (32), alkaline phosphatase (37, 39), and β-lactamase encoded by the *bla* gene (1) and, in *B. subtilis*, the secreted protein α-amylase (36). Studies of the temporal mode of processing indicate that the outer membrane OmpA as well as the periplasmic β-lactamase encoded by *ampC* are processed while nascent, providing indirect evidence of membrane association during synthesis (18), since leader peptidase is a well-characterized membrane protein (46).

We have chosen to represent the translocation step in our working model (Fig. 3) as more closely related to that in the membrane-triggered folding hypothesis than to that in the signal hypothesis: translocation is considered to be independent of elongation. Let us look in some detail at the data that led to that conclusion.

INDEPENDENCE OF TRANSLOCATION FROM ELONGATION

We first suspected that translocation of the exported polypeptides might be delayed relative to synthesis when Josefsson observed that for several proteins the removal of the leader sequence was a late event relative to synthesis (18). To discuss these experiments and the ones that follow, we must explain the technique developed to study nascent polypeptides. When an exponentially growing culture of *E. coli* is pulse-labeled and the solubilized cells are immunoprecipitated with antiserum specific for an exported protein, the immunoprecipitate contains radioactively labeled nascent polypeptides in addition to the precursor and mature species. However, analysis of the precipitate on a standard sodium dodecyl sulfate (SDS)-polyacrylamide gel will reveal only the precursor and mature species. The nascent chains are not visible. This is because nascent chains are a heterodisperse population with respect to molecular weight. Therefore, although they do migrate in the region of gel below the mature species, they do not form distinct bands. This problem can be overcome if, instead of attempting to analyze the intact nascent polypeptides, we study a proteolytic digest which will contain discrete peptide species. In our experiment we wanted to analyze the nascent chains of maltose-binding protein with respect to their length, so we first separated them according to molecular weight by SDS-polyacrylamide gel electrophoresis. The strip of gel containing the precursor, mature, and nascent forms of the protein was cut out along the migration dimension and placed

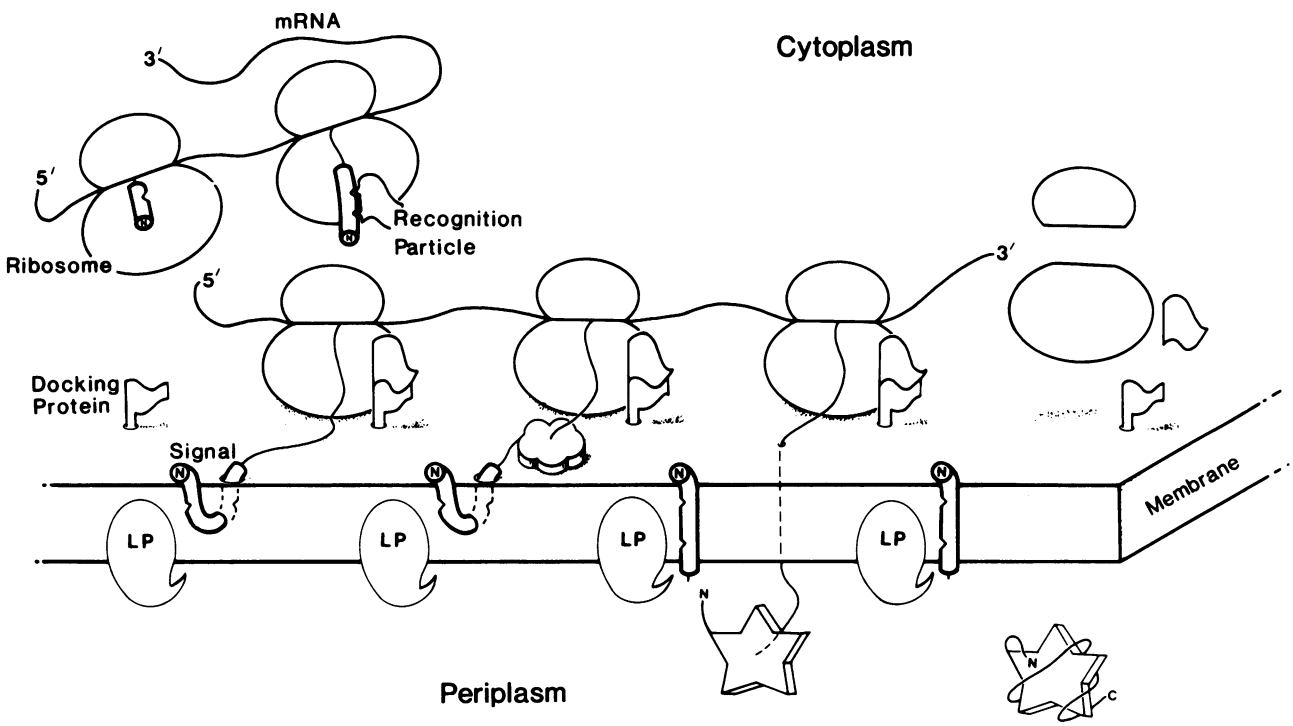

FIG. 3. Working model: export of a soluble protein through the cytoplasmic membrane of *E. coli* into the periplasmic space. LP, Leader peptidase. The flower and star represent unspecified conformations. The docking protein and leader peptidase are represented as membrane-associated proteins.

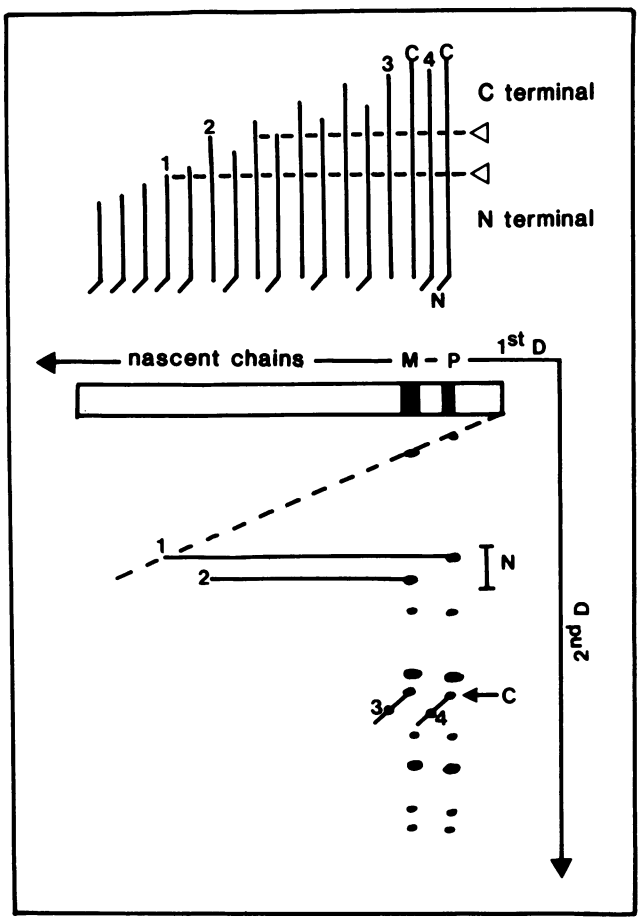

FIG. 4. Schematic representation of two-dimensional analysis of nascent polypeptides. See text for discussion. M, Mature species; P, precursor, D, dimension.

horizontally on the top of a second SDS-polyacrylamide gel. The nascent polypeptides in the gel strip were subjected to partial proteolysis (6), generating a reproducible pattern of large fragments. (A complete description of the technique can be found in Josefsson and Randall [19].) In this way, visible distinct peptides were generated from the invisible polydisperse nascent chains. Figure 4 shows the nascent chains represented diagrammatically above their position of migration in the first electrophoretic separation. Proteolysis is carried out in the gel before the second electrophoretic fractionation. Thus the peptides appear in the slab gel in a vertical array directly beneath the position of the nascent chain from which they were derived. To simplify discussion we will consider only those peptides that contain the amino or carboxyl terminus. These are generated by cleavage at sites represented schematically by the dashed lines. Consider first the peptide that contains the amino terminus (N terminal, Fig. 4). It is present among the proteolytic products of all nascent chains that are long enough to contain the complete peptide. Thus the peptide appears as a horizontal streak in the second dimension, starting at the position of the fully elongated precursor and extending to the main diagonal. Any material that migrates with the same apparent molecular weight in the first and second electrophoretic steps will lie on the main diagonal. Examination of the illustration shows that the streak representing the amino-terminal peptide is unique in that it is the only streak that fuses with the diagonal. That is because the shortest nascent chain which contains the peptide (no. 1, Fig. 4) is the one that is elongated precisely to the amino acid that is at the proteolytic cleavage site. All other peptides are smaller than the chains from which they are derived and migrate below the diagonal.

The peptide that contains the carboxyl terminus (C terminal, Fig. 4) is complete only in the fully elongated species, since even the longest nascent chain lacks the terminal amino acid residue. Thus this peptide does not form a horizontal streak at all, but rather appears as a diagonal streak. We can tell whether the peptide was derived from a nascent chain carrying the signal, or from one that had been matured, by examining the origin of the streak. If it fuses with a spot in the vertical array of peptides derived from the mature species (M), then the nascent chain had been matured. If it fuses with a peptide in the column under the position of precursor (P), then the nascent chain carried the signal sequence. In the case of maltose-binding protein, a distinct spot can be seen on each diagonal streak. Spot 3 represents a nascent chain elongated to the same amino acid residue at the carboxyl terminus as the nascent chain from which spot 4 was derived. However, spot 4 is to the right of spot 3 because it came from a nascent chain that carried the signal sequence at the amino terminus, whereas spot 3 came from a matured nascent chain. The spots on the streaks (no. 3 and 4) are derived from discrete species of nascent chains that accumulate transiently as a result of a drastic decrease in the rate of elongation at specific sites during synthesis of maltose-binding protein. The nonuniform rate of elongation of this protein was described earlier (33).

In all of our analyses we use spot 3 as an index of nascent chains that have been processed and spot 4 as an index of those that have not. Likewise, the streak numbered 1 is indicative of nascent chains that are not processed. The corresponding amino-terminal peptide from chains that have been processed migrates as a horizontal streak at a molecular weight lower by 2,500. As mentioned earlier, Josefsson used the two-dimensional peptide map to determine whether maltose-binding protein was processed while nascent. The presence of the matured amino terminus among the population of nascent chains showed that processing did occur cotranslationally (Fig. 5 shows a typical analysis of nascent maltose-binding protein). The unexpected result was that the streak representing the amino-terminal peptide from the mature species of maltose-binding protein did not extend to the diagonal. It first appeared at a position characteristic of nascent chains of molecular weight 31,000 (see point designated 2 in diagram [Fig. 4] and Fig. 5 for actual data). In other words polypeptides had reached 33,000 (31,000 + 2,000 signal) out of a total molecular weight of 41,000 before the signal was removed. We termed this the critical molecular weight for processing. Similar studies showed that arabinose-binding protein, alkaline phosphatase, and OmpA were also elongated to approximately 80% of the full length before processing was initiated (18).

The requirement of a critical size to initiate processing might be explained in several ways. Perhaps the processing enzyme, leader peptidase, does not recognize the precursor as a substrate until a substantial portion of the polypeptide has folded. We were able to eliminate this possibility for maltose-binding protein by demonstrating that nascent chains of maltose-binding protein could be processed in vitro at a molecular weight lower than the critical molecular weight observed in vivo. Purified leader peptidase obtained from W. Wickner was incubated with nascent maltose-binding protein elongated in vitro in the absence of membrane. Analysis

of the nascent chains (Fig. 6B and C) shows that the peptide representative of matured nascent chains was present along the entire molecular weight range from the fully elongated mature species to the diagonal (molecular weight, 26,000), whereas in vivo, as discussed above (Fig. 5 and 6A), the peptide first appeared at molecular weight 31,000 (i.e., derived from chains that were 33,000). We were not able to assess the processing of nascent chains of molecular weight below 26,000 since the peptide that we use as an index of matured chains itself has a molecular weight of 26,000. Nevertheless, it is clear that nascent chains were processed before reaching the critical molecular weight.

A similar result was obtained when membrane-bound polysomes were isolated and the nascent chains they carried were processed in vitro in the presence of the detergent Triton X-100 to activate the leader peptidase that was present in the membranes themselves (Fig. 6D). Again, matured nascent chains were present at molecular weight 26,000 (the limit of the assay; see above).

Thus it appeared that leader peptidase would process nascent chains of low molecular weight if it had access to them. Ito and Beckwith (15) provide further support for this conclusion with the finding that the signal sequence was removed from an amber fragment of maltose-binding protein that was less than half the full length.

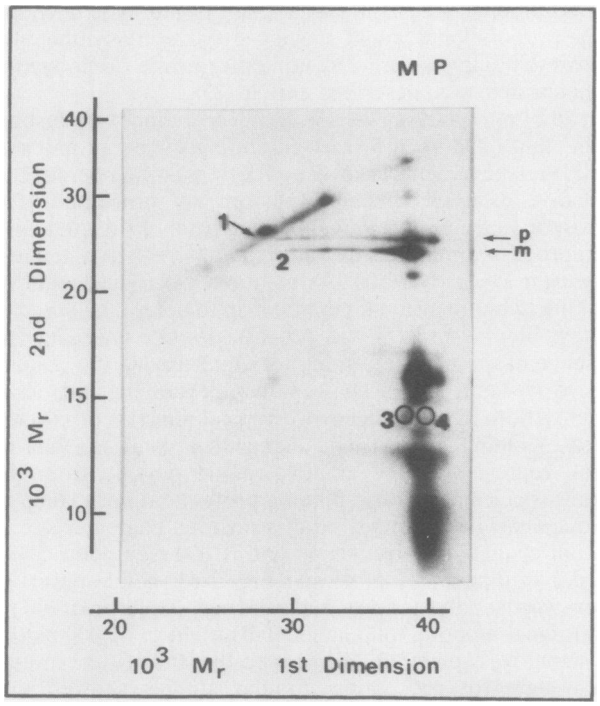

FIG. 5. Analysis of pulse-labeled nascent polypeptides of maltose-binding protein. A culture of exponentially growing *E. coli* (cell density, 2.5×10^8 cells per ml) was pulse-labeled for 15 s with 15 μCi of [^{35}S]methionine and treated to obtain immunoprecipitates of maltose-binding protein. The immunoprecipitated polypeptides were separated by SDS-10% polyacrylamide gel electrophoresis (first dimension). The section of the first-dimension gel lane including the region containing precursor (P), mature (M), and nascent maltose-binding protein was placed lengthwise on top of an SDS-15% polyacrylamide gel (second dimension) and subjected to limited proteolysis, using *Staphylococcus aureus* V8 protease. The procedure, including the labeling, immunoprecipitation, gel electrophoresis, and proteolysis, is given in detail in Josefsson and Randall (19). The data are represented diagrammatically in Fig. 4 and are discussed in the text.

It seemed likely, therefore, that processing was delayed because translocation itself was a late event. If translocation were rate limiting, then increasing the quantity of leader peptidase in the membrane should have no effect on rate of processing. A strain had been constructed in Wickner's

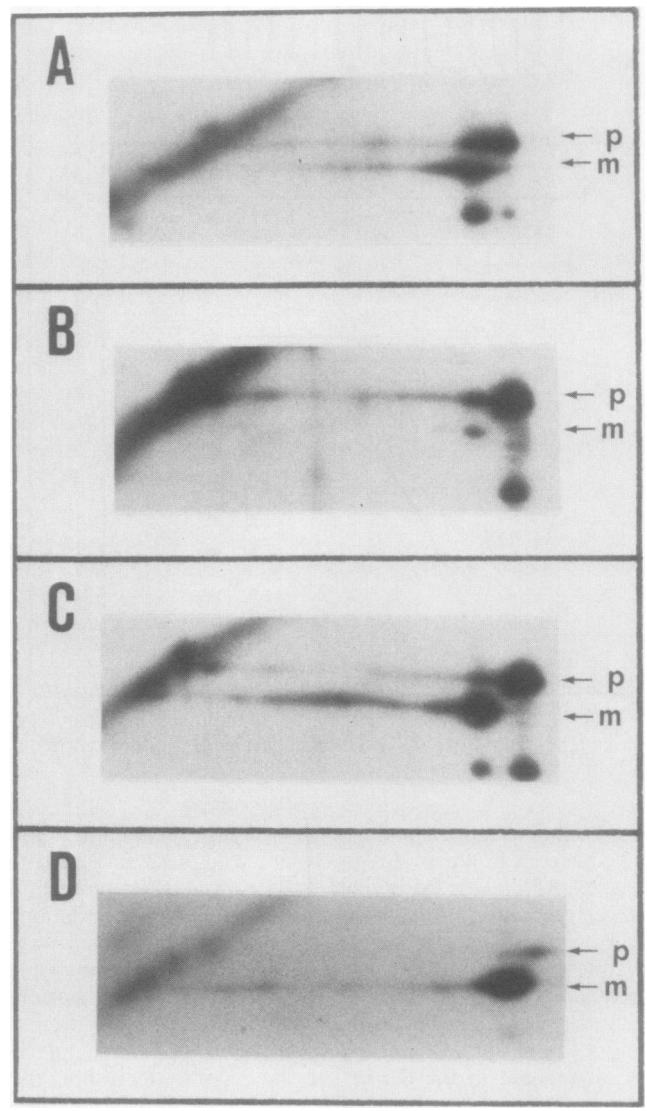

FIG. 6. Processing of maltose-binding protein in vivo and in vitro. The region of the two-dimensional analysis that contains the peptides derived from the amino terminus is shown: p, peptide derived from nascent chains carrying the signal sequence; m, peptide derived from nascent chains that have been matured. (A) Analysis of nascent chains labeled in vivo as described in the legend to Fig. 5. (B) Nascent chains elongated in vitro on free polysomes in the absence of membrane. (C) Nascent chains elongated in vitro on free polysomes in the presence of purified leader peptidase and 0.1% Triton X-100. (D) Nascent chains elongated in vitro on membrane-bound polysomes in the presence of 0.3% Triton X-100 to stimulate processing. Free and membrane-bound polysomes were prepared as described in reference 31 except that the culture was pulse-labeled for 20 s with [^{35}S]methionine immediately before harvest. Elongation in vitro was as described previously (31). Incubation was for 60 s at 35°C for membrane-bound polysomes. In the case of free polysomes (B, C) elongation was terminated at 2 min by addition of chloramphenicol (100 μg/ml) and incubation was continued for an additional 28 min at 35°C.

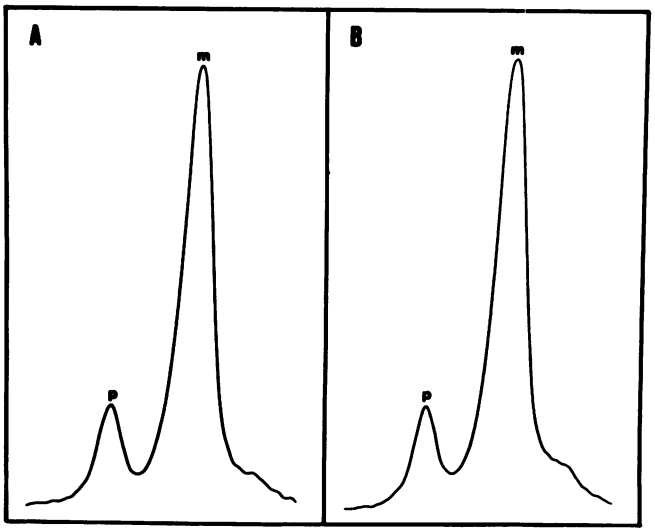

FIG. 7. Processing of maltose-binding protein in a strain which overproduces leader peptidase. Strains JA200 harboring either plasmid pTD101 (B) that carries the structural gene for leader peptidase (9) or a related plasmid, pTD126 (A), which has the gene for leader peptidase deleted was pulse-labeled with [^{35}S]methionine for 15 s and processed for immunoprecipitation with antiserum to maltose-binding protein. The precipitate was analyzed by SDS-10% polyacrylamide electrophoresis. The autoradiogram of the dried gel was scanned with a Helena Laboratories Quick Scan. Densitometer tracings of the relevant portion of the gel are shown. The position of precursor maltose-binding protein is indicated by p and that of mature maltose-binding protein is shown by m. Portions of the same cultures were analyzed in vitro for leader peptidase activity by cleavage of radioactive procoat to coat as described before (49). Extracts of JA200(pTD101) were 10 times as active in the assay as extracts of JA200(pTD126).

laboratory (9) that overproduced leader peptidase. A higher activity was expressed in vivo as shown by an increase in the rate of maturation of M13 coat protein.

Eva Murén (this laboratory) in collaboration with Marilyn Rice in Wickner's group examined processing of maltose-binding protein in this strain. Although they demonstrated a 10-fold overproduction of leader peptidase by assaying processing of the precursor of M13 coat protein in vitro, they saw no effect in vivo on the rate of processing of maltose-binding protein, thus providing evidence that processing was not rate limiting (Fig. 7).

Therefore, we turned to direct investigation of the time of the translocation event. We could determine the time of translocation by using accessibility of nascent chains to externally added protease. Nascent chains would be protected as long as they remained on the cytoplasmic side of the membrane: translocation would render them sensitive.

A complete description of these investigations, including the relevant controls, is found in Randall (30). Here we briefly outline the experimental design (Fig. 8). Nascent chains were pulse-labeled with [^{35}S]methionine. The cells containing the labeled polypeptides were converted to spheroplasts so that the external surface of the cytoplasmic membrane would be accessible to the protease that was added. After the exposed polypeptides had been digested, the protease was inactivated and the polypeptides related to maltose-binding protein were immunoprecipitated. The nascent chains were analyzed by the two-dimensional gel electrophoresis technique. A typical example of this type of experiment is shown in Fig. 9. The population of nascent chains immunoprecipitated from a control pulse-labeled culture contained both precursor and matured species as indicated by the presence of the amino-terminal and carboxyl-terminal peptides characteristic of these species (Fig. 9A, streaks p and m, spots 3 and 4). The map of nascent chains immunoprecipitated from the spheroplasts that had been treated with protease (Fig. 9B) shows that the peptides characteristic of the matured nascent chains (streak m, spot 3) were absent whereas those characteristic of precursor species (streak p, spot 4) remained. It should be noted that the fully elongated mature maltose-binding protein was resistant to proteolytic digestion. Such resistance is a common feature among periplasmic proteins. Control experiments demonstrated that the precursor species of nascent chains were not inherently resistant to digestion, but were degraded when made accessible to protease (30). The simplest interpretation of these data is that nascent chains remain on the cytoplasmic side of the membrane until time of maturation. Since maturation of maltose-binding protein is a late event, beginning after the polypeptide is 80% complete, these observations are inconsistent with a translocation mechanism that is coupled to elongation.

In addition, it has been demonstrated in vivo that at least three proteins, M13 coat protein (10), β-lactamase (21), and ribose-binding protein (30), are not only processed post-translationally but in fact are translocated after synthesis is complete. Clearly these proteins are not translocated in a continuous fashion that is coupled to elongation.

If one is allowed to assume, as seems to be the case, that processing is an index of translocation, then transfer of polypeptides independent of elongation also pertains to many other proteins. These include all proteins that are processed post-translationally as well as proteins that are processed while nascent only after achieving a length significantly greater than that required to span the membrane in a linear form. The former set includes proteins, too numerous

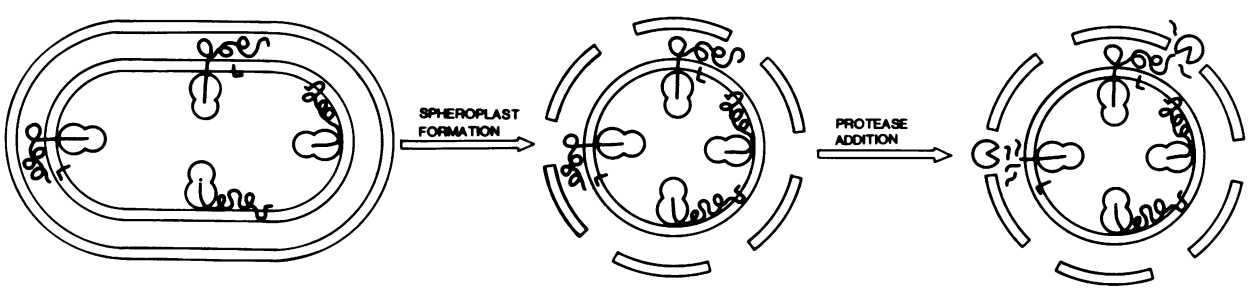

FIG. 8. Experimental design for assessing translocation of nascent chains, using accessibility to proteinase K.

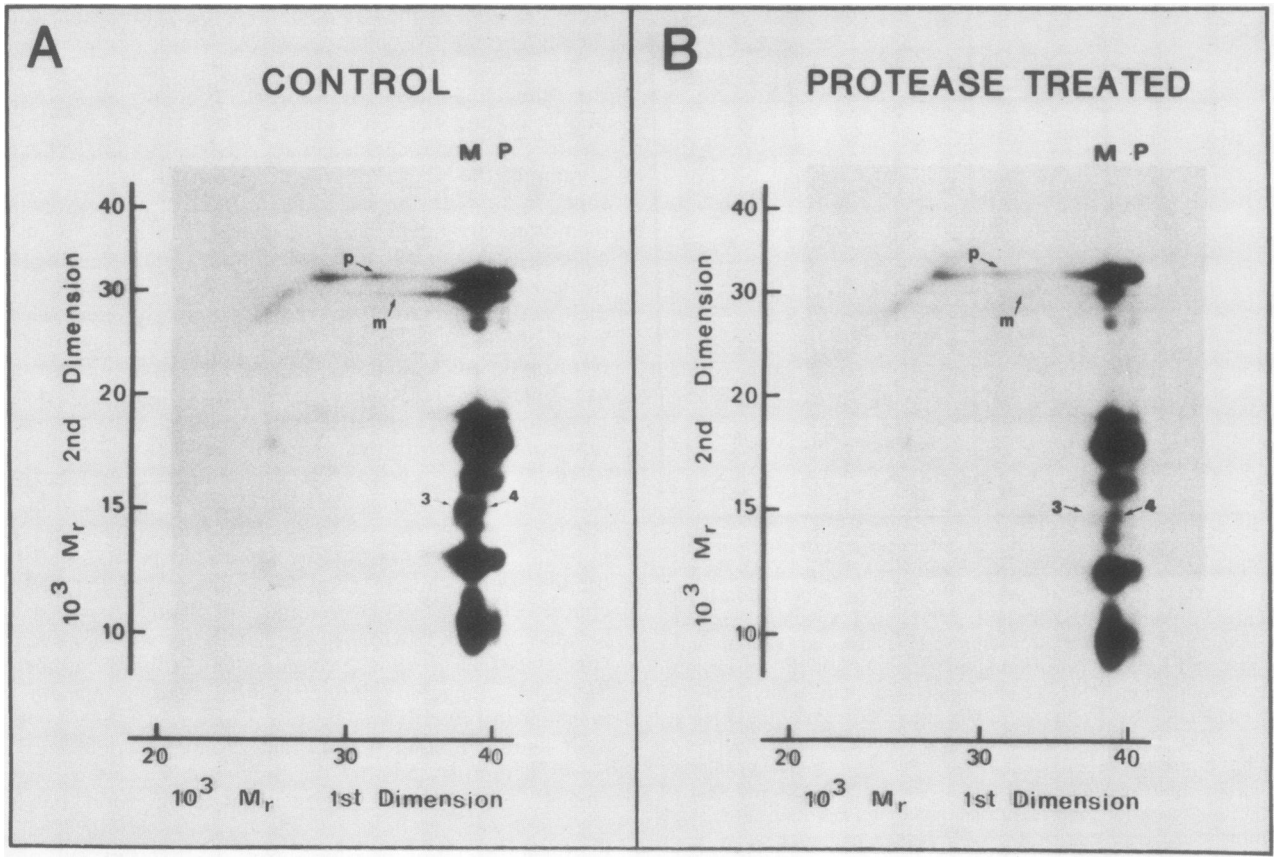

FIG. 9. Accessibility to proteinase K of nascent chains of maltose-binding protein. Generation of the two-dimensional peptide pattern derived from polypeptides immunoprecipitated with antiserum to maltose-binding protein was as described in the legend to Fig. 5. Cultures were pulse-labeled (15 s) and converted to spheroplasts as described below. One portion served as a control (A) to show the species of nascent chains that were present, and a second portion was treated with proteinase K (25 μg/ml) to assess the susceptibility of the nascent chains to proteolytic digestion. After a 30-min incubation on ice, the protease inhibitor phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM. The samples were processed for immunoprecipitation and analysis of nascent chains as given (in reference 19). This experiment differs from those described by Randall (30) as follows: labeling with [^{35}S]methionine was terminated at 15 s by pouring 1.5 ml of culture (2.5 × 10^8 cells per ml) onto frozen, crushed medium containing carbonyl cyanide *m*-chlorophenylhydrazone (50 μM). After centrifugation the cells were suspended in 0.375 ml of 0.1 M Tris-acetate (pH 8.2)–0.5 M sucrose–5 mM EDTA. Lysozyme was added (30 μl of 2 mg/ml) followed by addition of 0.375 ml of ice-cold water. After a 10-min incubation, $MgSO_4$ (final concentration, 20 mM) was added to stabilize the spheroplasts. All other operations were as described previously (30). M, Mature species; P, precursor. Note: The scale on the ordinate is misaligned; see Fig. 5 for the correct molecular weights.

to list, for which a precursor form has been demonstrated in vivo (for reviews, see references 14 and 27 and Bankaitis et al., in press), and the latter includes arabinose-binding protein, alkaline phosphatase, and the OmpA protein (18). There is no evidence for continuous linear extrusion coupled to elongation of any bacterial exported protein.

SUMMARY

The phenomenon of export is complex process comprising at least four heuristically separable, though not necessarily mechanistically separable, steps: recognition, translocation, proteolytic processing, and release. Until data become available to the contrary, we shall assume in our working model (Fig. 3) that recognition proceeds as set forth in the modified version of the signal hypothesis (25). After recognition and membrane association, the polypeptide is elongated at the membrane. After synthesis of a substantial portion of the polypeptide, translocation proceeds by a mechanism, as yet unknown, which may involve participation of membrane proteins. Such proteins might be the products of one or more of the genes already characterized as involved in export (27; Bankaitis et al., in press). The model has features in common with that proposed by Koshland et al. to explain the export of β-lactamase in *Salmonella typhimurium* (22). The important different feature of this scheme is that synthesis at the membrane is required to ensure that the polypeptide does not fold in the cytoplasm into a conformation that is incompatible with translocation. Immediately after translocation, the leader sequence is proteolytically removed by leader peptidase. Since the source of energy for transfer is not defined, one might suppose that the cleavage is a vectorial reaction. However, this seems unlikely, since translocation of precursors that have not been cleaved has been demonstrated for β-lactamase (22) and the membrane proteins, lipoprotein (23), and M13 coat protein (47). A candidate for the driving energy is the electrochemical membrane potential. Although this is required for export of protein in *E. coli* (7, 8, 11, 13, 48), it must be appreciated that an electrochemical membrane potential is not necessarily a motive force but might play an indirect role. An alternative candidate for the motive force is conformational change of the exported protein. The final stage of export is release of the soluble protein from the membrane on the extra-

cytoplasmic side. Release is considered to be a defined step, since it is known that mutant forms of β-lactamase (20) and ribose-binding protein (24), even though proteolytically matured, are not released.

An important question is whether or not procaryotes and eucaryotes use the same mechanism of export. Clearly there are striking similarities since bacterial proteins synthesized in eucaryotic cell-free systems can be transported into the lumen of microsomes (29), cloned eucaryotic proteins expressed in *E. coli* are secreted (38), and the bacterial plasmid-encoded β-lactamase was secreted when introduced into either *Saccharomyces cerevisiae* (34) or *Xenopus* oocytes (45). The single most obvious difference is that the requirement for an energized membrane, demonstrated in *E. coli*, does not exist in eucaryotes for protein secretion. This discrepancy might not reflect a fundamental difference in mechanism but might be due only to the drastic difference in structure between the envelope of *E. coli* and the membrane of the endoplasmic reticulum. Since there is no evidence to the contrary, and in light of the analogies between the systems, it seems likely that the mechanisms are basically the same.

ACKNOWLEDGMENTS

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