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Trigger factor: A soluble protein that folds pro-OmpA into a membrane-assembly-competent form

(*in vitro* protein synthesis/leader peptide/protein folding/protein translocation)

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ABSTRACT **Pro-OmpA that is synthesized *in vitro* can assemble into bacterial inner membrane vesicles in the presence of ATP and NADH. We have purified pro-OmpA to determine which additional soluble proteins are necessary for its membrane assembly. [^{35}S]Pro-OmpA was bound to Sepharose-linked antibody to OmpA, then eluted with 8 M urea and chromatographed on an anion-exchange resin in 8 M urea. This pro-OmpA is purified 2000-fold and is radiochemically pure. After dialysis, it is soluble but incompetent for membrane assembly. Addition of an *Escherichia coli* cytoplasmic fraction (S100) to the assembly reaction does not allow translocation. However, when S100 is added to pro-OmpA prior to dialysis, full assembly competence is restored, suggesting that a soluble factor, termed "trigger factor," triggers the folding of pro-OmpA into an assembly-competent form as the urea is removed. We noted that, prior to the last purification step, the immunoaffinity-purified pro-OmpA was partially competent for membrane assembly without addition of trigger factor. To test whether trigger factor had bound to the antibody column by means of its association with pro-OmpA, the crude pro-OmpA was acid-denatured prior to immunoadsorption. In this experiment, the trigger factor did not bind to the anti-OmpA column, and S100 was required for renaturation of this [^{35}S]pro-OmpA. As suggested by this experiment, the crude [^{35}S]pro-OmpA was in a complex with other proteins. Sedimentation velocity studies showed that the trigger factor has an apparent molecular weight of ≈60,000. We propose that it is required for translocation-competent folding of pro-OmpA and other precursor proteins.**

Protein translocation across the bacterial plasma membrane, the eukaryotic rough endoplasmic reticulum, and mitochondrial membranes has several shared features (1). Translocation requires energy, either as high-energy phosphate, an electrochemical potential, or both. Most proteins require an amino-terminal leader peptide for translocation, though this sequence can reside within the mature protein. Translocation is usually rapid, but even for the endoplasmic reticulum it is not necessarily or mechanistically coupled to ongoing protein synthesis (2). However, the mechanisms for protein translocation remain largely undefined. Biochemical studies have identified leader peptidases that can remove cleavable leader sequences. Translocation into the endoplasmic reticulum requires the signal-recognition particle and docking protein, which form a protein-targeting system for that organelle (3). Genetic studies have identified several bacterial secretion (*sec*) proteins (4), which may target proteins to the bacterial cell surface.

Precursors of bacterial cell surface proteins, synthesized *in vitro*, will assemble posttranslationally into inverted plasma membrane vesicles (5, 6) in the presence of ATP and a membrane electrochemical potential (7, 8). This makes it possible to fractionate and reconstitute this translocation reaction to determine the necessary components. Muller and Blobel (9) discovered that a 12S factor is needed for protein translocation across inner membrane vesicles but not for polypeptide synthesis.

We have purified pro-OmpA, the precursor of *o*uter *m*embrane *p*rotein A, to determine if any other soluble proteins catalyze the translocation of this precursor protein across the plasma membrane of *Escherichia coli*. We find that an activity in the cytosol (S100) fraction is necessary for translocation. This "trigger factor," with an apparent molecular weight of ≈60,000, appears to act during the folding of the protein, yet its effects are not coupled to translation. The trigger factor allows pro-OmpA to fold into an assembly-competent form.

MATERIALS AND METHODS

Materials and Bacterial Strains. *E. coli* RNA polymerase and proteinase K were from Boehringer Mannheim. Phenylmethylsulfonyl fluoride and bovine serum albumin (BSA) were from Sigma. CNBr-activated Sepharose 4B and FPLC Mono Q column were from Pharmacia. DE52 DEAE-cellulose was from Whatman. "Translabel," a mixture of 85% [^{35}S]methionine and 15% [^{35}S]cysteine (1000 Ci/mmol; 1 Ci = 37 GBq), was from ICN. Carrier-free ^{125}I was from Amersham. S100 and ribosomes were prepared from *E. coli* strain Q13 (*met, tyr, rna,* Hfr). Inverted inner membrane vesicles were prepared from *E. coli* strain D10 (*rna-10, relA1, spoT1, metB1*) as described by Rhoads *et al.* (10).

Cell-Free Protein Synthesis. ^{35}S-labeled pro-OmpA was synthesized by a modification of the procedure of Gold and Schweiger (11), as described by Bacallao *et al.* (12). Plasmid DNA containing the *omp9* gene was prepared as described by Geller *et al.* (8).

Pro-OmpA Affinity Column. IgG was prepared from rabbit antiserum to OmpA (13) and coupled to CNBr-activated Sepharose 4B.

***In vitro* Protein Translocation.** Translocation of pro-OmpA into *E. coli* inverted inner membranes was assayed by accessibility to added protease, as described by Bacallao *et al.* (12).

Cytosolic Extract (S100). S100 was prepared by a modification of the procedure of Gold and Schweiger (10). Briefly, a frozen 50% (wt/wt) suspension of *E. coli* Q13 in 50 mM Tris Cl, pH 8.0/10% sucrose was thawed and disrupted by blending with glass beads. After low-speed centrifugation, the supernatant was centrifuged at 100,000 × g_{av} for 60 min. The high-speed supernatant was applied to a DE52 column equilibrated in 22 mM NH_4OAc/10 mM Tris Cl, pH 7.5/10 mM $Mg(OAc)_2$/1 mM dithiothreitol (buffer A), eluted with 350 mM NH_4OAc in buffer A, and stored at −70°C. For

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Abbreviations: BSA, bovine serum albumin; S100, cytoplasmic fraction (100,000 × *g* supernatant).

pro-OmpA purification experiments, S100 was labeled with ^{125}I by lactoperoxidase (14).

Purification of Pro-OmpA. *Step I.* [^{35}S]Pro-OmpA was synthesized in a 750-μl cell-free synthesis reaction mixture with 1.5 mCi of Translabel. ^{125}I-labeled S100 (100 μl; 7.8 × 10^6 cpm) was added and the mixture was centrifuged in a Beckman Airfuge (105,000 × *g*, 15 min, 2°C) to remove polysomes. *Step II.* The resulting supernatant was applied to a 1.0 × 3.8-cm anti-OmpA IgG-Sepharose 4B affinity column equilibrated in 66 mM Tris Cl/25 mM NH_4Cl/10 mM $Mg(OAc)_2$/2 mM dithiothreitol, pH 7.6 (buffer B). The column was washed with one column volume of 0.5 M NaCl/1% (vol/vol) Triton X-100/buffer B, then with four column volumes of buffer B. Bound proteins were eluted from the column by 50 mM Tris Cl, pH 8.0/2 mM dithiothreitol/8 M urea (buffer C) and peak fractions of pro-OmpA were pooled. *Step III.* Step II pro-OmpA (1.5 ml) was applied to a 1.0-ml FPLC Mono Q anion-exchange column equilibrated in buffer C. Pro-OmpA appeared in flow-through fractions and was pooled. All procedures following the synthesis were performed at 2°C. [^{35}S]Pro-OmpA was quantified by liquid scintillation counting of immunoprecipitated pro-OmpA for step I or total radioactivity for steps II and III. The efficiency of immunoprecipitation of pro-OmpA was measured with step III material (32%), and the step I measurement was adjusted on the assumption that it had the same immunoprecipitation efficiency. Total ^{125}I-labeled protein content was quantified by γ-radiation counting. (See Table 1.)

Other Methods. Protein concentration was determined by the method of Lowry *et al.* (15). Samples were analyzed by $NaDodSO_4$/PAGE and fluorography (16). Where indicated, samples were immunoprecipitated with anti-OmpA serum (17).

RESULTS

To allow sensitive assay of total soluble protein during the purification of [^{35}S]pro-OmpA, an aliquot of S100 was radioiodinated. The protein concentration of the initial *in vitro* protein synthesis reaction is 2 mg/ml, and immunoblot analysis showed that the reaction produced 1 μg of pro-OmpA per ml. Therefore a purification of ≈2000-fold would yield nearly homogeneous pro-OmpA. During the purification, [^{35}S]pro-OmpA was assayed either by fluorography (Fig. 1) or by liquid scintillation counting of immunoprecipitates (step I) or total radioactive proteins (steps II and III) (Table 1). The total protein content was determined by γ-radiation counting (Table 1).

[^{35}S]Pro-OmpA was synthesized in a cell-free reaction. ^{125}I-labeled S100 was added (Table 1) and the mixture was centrifuged to remove polysomes. This material is step I pro-OmpA (Fig. 1, lane 1, and Table 1). The supernatant was applied to a column of Sepharose 4B bearing covalently bound IgG antibodies to OmpA. Pro-OmpA was eluted from the column with 8 M urea (step II pro-OmpA; Fig. 1, lane 2, and Table 1). The affinity-purified material was further chromatographed through a Mono Q anion-exchange column equilibrated with 8 M urea. Pro-OmpA appeared in the flow-through fractions (step III pro-OmpA; Fig. 1, lane 3, Table 1). Step III pro-OmpA is radiochemically pure and may be nearly chemically pure, as judged by its relative abundance prior to purification.

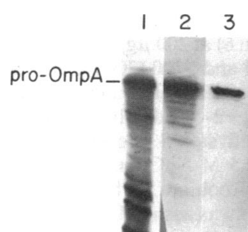

FIG. 1. Isolation of radiochemically pure pro-OmpA. Fractions containing equal quantities of radioactive pro-OmpA from steps I, II, and III (see *Materials and Methods* and Table 1) were analyzed by $NaDodSO_4$/PAGE and fluorography in lanes 1, 2, and 3, respectively.

Table 1. Purification of pro-OmpA

Step	pro-OmpA, ^{35}S cpm	Protein, ^{125}I cpm	Yield, %	Purification
I. *In vitro* synthesis	2.5×10^7	7.8×10^6	100	1
II. Immunoaffinity	3.0×10^6	2.2×10^3	12	42
III. Anion exchange	4.7×10^5	77	1.9	1924

The translocation competence of pro-OmpA was assayed at each step of the purification. Inverted inner membrane vesicles translocated and processed 25% of the pro-OmpA synthesized in the cell-free reaction (Fig. 2*A*, lane 1). The OmpA was protected from added protease, and this assembly required ATP and NADH (Fig. 2*A*, lanes 2–4). Pro-OmpA and OmpA are readily digested by protease when membrane integrity is disrupted (17). Following affinity purification, an aliquot was dialyzed to remove the urea. BSA was added to the sample prior to dialysis to limit the adsorption to the dialysis bag. Control experiments showed that BSA, added before or after dialysis of pro-OmpA, does not inhibit or activate translocation of pro-OmpA across inner membrane vesicles (unpublished data). Dialyzed step II pro-OmpA showed a limited competence for energy-dependent translocation (6.2%) without the addition of any other soluble factors (Fig. 2*B*, lanes 2–4). However, dialyzed step III pro-OmpA (Fig. 2*C*, lane 1) was unable (<1%) to translocate into inner membrane vesicles (Fig. 2*C*, lanes 2–4).

To determine whether the inability of purified pro-OmpA to translocate was due to the absence of a required translocation factor, we added S100 to the purified pro-OmpA prior to mixing with inner membrane vesicles. Although pro-OmpA could be recovered as a soluble protein after the dialysis, <1% was translocated and processed in the presence of S100 (Fig. 3, lane 2). However, when pro-OmpA was dialyzed in the presence of 200 μg of S100 protein per ml (Fig. 3, lane 4), 11% was capable of translocation (lanes 5 and 6). As shown below (Fig. 6*A*), this is in the range of linear response to added S100 and is no more than one-third the maximal yield of processed, sequestered OmpA seen with higher amounts of S100. These data indicate that a soluble factor is required for translocation. Purified thioredoxin, which has been reported to affect the folding of other proteins (18), did not substitute for S100 in renaturing pro-OmpA (unpublished data). S100 that had been mixed with urea and dialyzed was still not competent to restore translocation to pro-OmpA that had been dialyzed separately (unpublished data). Since the S100 must be added to the pro-OmpA prior to removal of urea, it appears that the function of this factor is to allow proper folding of the precursor protein, possibly by forming a complex. We refer to the active component of S100 as the "trigger factor," since it triggers membrane-assembly-competent folding (19).

Because the pro-OmpA eluted from the affinity column (step II) retained a limited competence for translocation following dialysis, it seemed possible that pro-OmpA and the trigger factor bound to the antibody column as a complex. Addition of 8 M urea to dissociate pro-OmpA from the column might have disrupted the complex between pro-OmpA and the trigger factor. Effective separation of the two components was obtained by anion-exchange chromatography in 8 M urea (step III). To test this interpretation of the data, we employed an independent method to dissociate a possible complex and separate pro-OmpA and the trigger factor. Pro-OmpA was synthesized in an *in vitro* synthesis reaction and centrifuged as above (step I). Trichloroacetic acid was added to the reaction to disrupt existing intermo-

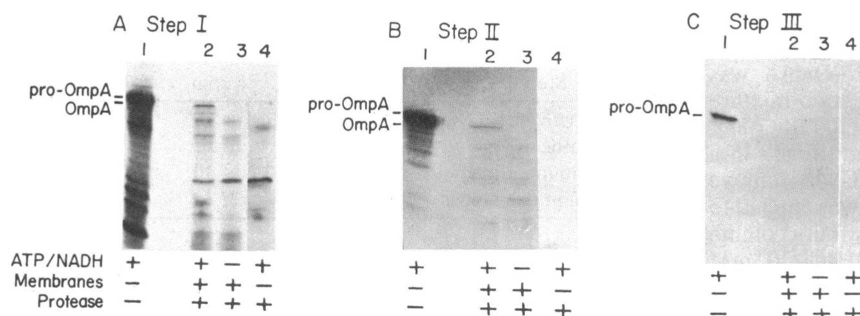

FIG. 2. *In vitro* translocation competence of pro-OmpA. Step I pro-OmpA was exchanged into buffer B as described (11). Aliquots (500 μl) of step II and step III pro-OmpA were dialyzed against four 1-liter portions of buffer B (2 hr per portion) at 2°C. Aliquots (50 μl) of [^{35}S]pro-OmpA (in buffer B) from steps I, II, and III (lanes 1) were incubated with membranes, ATP, and NADH as indicated. Proteinase K was subsequently added (lanes 2–4) to assay for translocation.

lecular complexes. The acid precipitate was resuspended in acetone, again collected by centrifugation, and then dissolved in 1% $NaDodSO_4$ at 95°C. After dilution with a buffer containing Triton X-100, the proteins were applied to the anti-OmpA affinity column. Pro-OmpA was eluted from the column with 8 M urea, mixed with either S100 or BSA, dialyzed to remove the urea, and tested for translocation (Fig. 4). If S100 was not present during the dialysis of this pro-OmpA, <1% was capable of energy-dependent translocation (Fig. 4*A*, lanes 2 and 3), even if the S100 was added prior to incubation with inner membrane vesicles (lanes 4 and 5). When urea-denatured pro-OmpA was mixed with S100 and dialyzed (lane 6), 14% of the pro-OmpA was capable of energy-dependent translocation (lanes 7–10). Step II pro-OmpA that had not been denatured prior to affinity chromatography on the antibody column was only stimulated 2-fold by S100 during its renaturation [Fig. 4*B*, 5% without S100 (lane 2) vs. 10% with S100 (lane 7)]. These results suggest that the trigger factor is required during the folding of pro-OmpA, perhaps for formation of a complex that confers assembly competence.

The proteins in the crude *in vitro* synthesis reaction were sized by constant velocity centrifugation (Fig. 5*A*) to further test the possibility of pro-OmpA being in a complex. Radioactive pro-OmpA sedimented with an apparent molecular weight of ≈160,000, whereas the molecular weight of pro-OmpA is 39,059 (20). The considerable discrepancy between the observed and predicted molecular weights suggests that the pro-OmpA synthesized in the reaction is in a multiprotein complex, either with itself or with other proteins. After gradient centrifugation, the pro-OmpA (Fig. 5*B*, lane 1) retained its capacity to translocate into membrane vesicles and be processed to mature OmpA that was inaccessible to external protease (lane 2). Further studies are necessary to establish whether the trigger factor is part of this complex, the stoichiometry of trigger factor and pro-OmpA, and whether other factors are present.

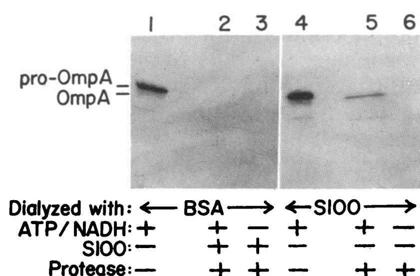

FIG. 3. S100 restores the translocation of pro-OmpA into inner membrane vesicles. Aliquots (200 μl) of step III pro-OmpA were mixed with 40 μg of either BSA (lanes 1–3) or S100 proteins (lanes 4–6) and dialyzed against four 200-ml portions of buffer B. Membrane-assembly assays were performed in the presence of S100 (200 μg/ml) and ATP plus NADH, as indicated, and membranes (lanes 2, 3, 5, and 6). Samples were incubated and assayed for translocation by addition of proteinase K.

The trigger-factor activity is proportional to the amount of S100 added during the dialysis step (Fig. 6*A*). Quantification of the fluorograph bands by densitometer scanning gave relative units of 4.5, 7.3, 12.3, and 0.4 for lanes 1–4, respectively. The observed concentration dependence allows accurate assaying of this activity. To determine whether the trigger factor is a specific protein, we fractionated the proteins of the S100 by glycerol gradient velocity sedimentation. The protein concentration of each gradient fraction was measured, and an equal amount of protein (16 μg) from each was mixed with purified pro-OmpA. After dialysis, samples were incubated with inner membrane vesicles in the presence of ATP and NADH. Translocation was assayed by protection from externally added protease (Fig. 6*B*). A peak

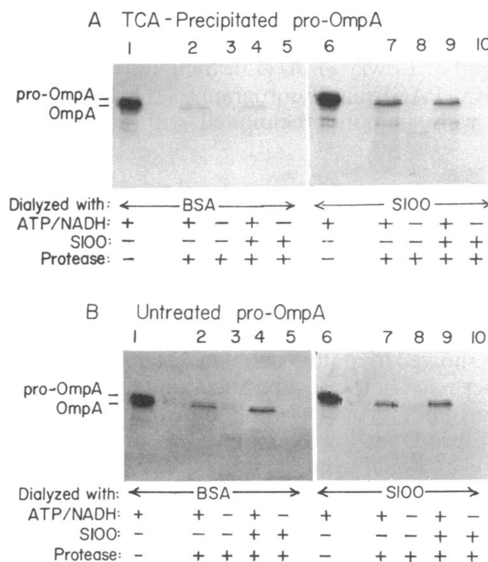

FIG. 4. Acid disruption of a pro-OmpA–trigger factor complex. Seven hundred microliters of step I [^{35}S]pro-OmpA was prepared as described in *Materials and Methods*. (*A*) Trichloroacetic acid (TCA) was added [final concentration 10% (wt/vol)] to 350 μl of the step I [^{35}S]pro-OmpA. The resulting precipitate was collected by centrifugation (5 min, Brinkmann microcentrifuge), suspended in 1.0 ml of cold acetone, and again collected by centrifugation. The pellet was dissolved in 50 μl of 50 mM Tris Cl, pH 8.0/2 mM dithiothreitol/1% $NaDodSO_4$, heated to 95°C for 3 min, cooled to room temperature, and then diluted up to 1 ml with buffer B containing 0.5 M NaCl and 1% Triton X-100. This solution was applied to the immunoaffinity column, and [^{35}S]pro-OmpA was eluted with 8 M urea (buffer C). Aliquots (300 μl) of eluted pro-OmpA were dialyzed in the presence of either BSA (lane 1) or S100 protein (lane 6) at 200 μg/ml. Samples (50 μl) were mixed with ATP plus NADH and S100 (200 μg/ml), as indicated. Samples were incubated with inner membrane vesicles, followed by digestion by proteinase K (lanes 2–5 and 7–10). (*B*) Three hundred fifty microliters of the untreated step I pro-OmpA was mixed with 1 ml of buffer B containing 0.5 M NaCl and 1% Triton X-100 and applied to the immunoaffinity column. [^{35}S]Pro-OmpA was eluted and dialyzed, and translocation was assayed as described for *A*.

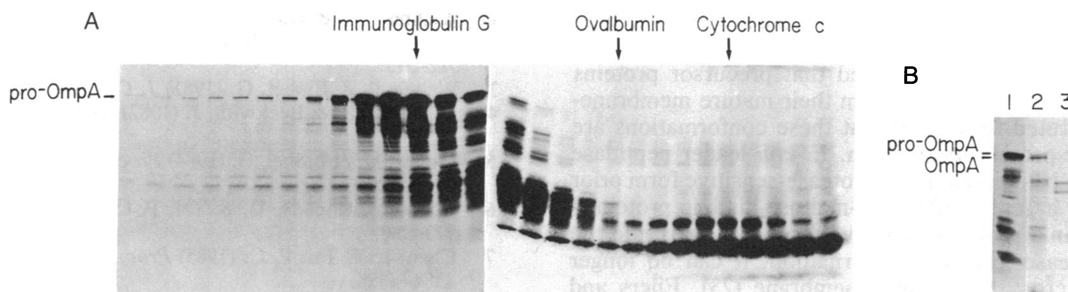

FIG. 5. Sedimentation of pro-OmpA as a complex. (*A*) Three hundred microliters of the ^{35}S-labeled *in vitro* synthesis reaction mixture was centrifuged (105,000 × *g*, 15 min, 2°C, Beckman Airfuge) to remove polysomes and applied to a 3.3-ml, 15–30% (vol/vol) glycerol gradient in buffer B. Molecular weight marker proteins (IgG, ovalbumin, and cytochrome *c*, each at 4 mg/ml, in 300 μl) were applied to an identical gradient. The gradients were centrifuged at 40,000 rpm in a Beckman SW60 rotor for 41 hr at 2°C. Fractions were collected, and aliquots were analyzed by NaDodSO$_4$/PAGE and fluorography. Molecular weight marker proteins were visualized by Coomassie blue staining. (*B*) ^{35}S-labeled pro-OmpA from the glycerol gradient peak was assayed for assembly into inner membrane vesicles as described in *Materials and Methods*. Lane 1: no vesicles or protease treatment. Lane 2: after membrane assembly, the sample was mixed with proteinase K and digestion was continued for 13 min at 0°C. Lane 3: as in lane 2, but digestion was in the presence of 1% octyl glucoside.

of specific activity for the trigger factor was found in gradient fraction 7, corresponding to an apparent molecular weight of ≈60,000. The specific activity of trigger factor in gradient fraction 7 was enriched 4.5-fold with respect to S100.

The availability of radiochemically pure [^{35}S]pro-OmpA as a translocation substrate also allowed us to develop a rapid and quantitative assay for *in vitro* protein translocation. OmpA that is inside sealed membrane vesicles is inaccessible to added protease, whereas pro-OmpA that has not translocated is accessible for degradation. After incubation of the translocation reaction with protease, the only ethanol-insoluble radioactivity remaining is due to the translocated, mature OmpA protein. In a typical translocation reaction, 2200 cpm of ethanol-insoluble [^{35}S]pro-OmpA was incubated with inner membrane vesicles in the presence of ATP and NADH and then digested with proteinase K. This procedure yielded 1050 ethanol-insoluble cpm. When either membranes or ATP and NADH were omitted, only 260 ethanol-insoluble cpm remained. This rapid and accurate assay for translocation will be a valuable tool for further fractionation of *in vitro* translocation reactions.

DISCUSSION

We have purified pro-OmpA 2000-fold from an *in vitro* protein synthesis reaction mixture in order to identify other components in the soluble fraction that are involved in its membrane assembly. We now report the existence of such a factor, which acts during the renaturation of pro-OmpA from the (urea) denatured state. This factor is sensitive to protease digestion but not to pancreatic ribonuclease (unpublished data). It may form a complex with pro-OmpA that triggers its folding into an assembly-competent form, although further studies are needed to establish this point. Since correct protein folding during membrane assembly was emphasized in the membrane trigger hypothesis (19), we term this protein the trigger factor. We do not know whether trigger factor might induce a specific conformation of pro-OmpA or just prevent pro-OmpA from folding into a particularly assembly-incompetent form. Our studies indicate that newly synthesized pro-OmpA is in a discrete complex and that it will bind to the antibody column as a complex unless it is denatured. During dialysis with purified, denatured pro-OmpA, trigger factor catalyzes the conversion of pro-OmpA to an assembly-competent form. This provides a sensitive assay that may be used to isolate this protein. Sedimentation velocity analysis indicates that trigger factor has an apparent molecular weight of ≈60,000, which suggests that it is distinct from the 12S (M_r ≈ 220,000) factor of Muller and Blobel (9) or the M_r 90,000 dalton SecA protein (21). Assays of trigger factor in extracts of SecA-deficient strains have not yet revealed any deficiency (unpublished data). We do not know whether trigger factor acts catalytically, whether it is one protein or more, whether its activity during the renaturation of pro-OmpA from urea faithfully reflects an *in vivo* role in the initial folding of this protein, or other aspects of the mechanism of its action. Purification of the trigger factor and the isolation of its gene are essential to biochemically evaluate the mechanism of its

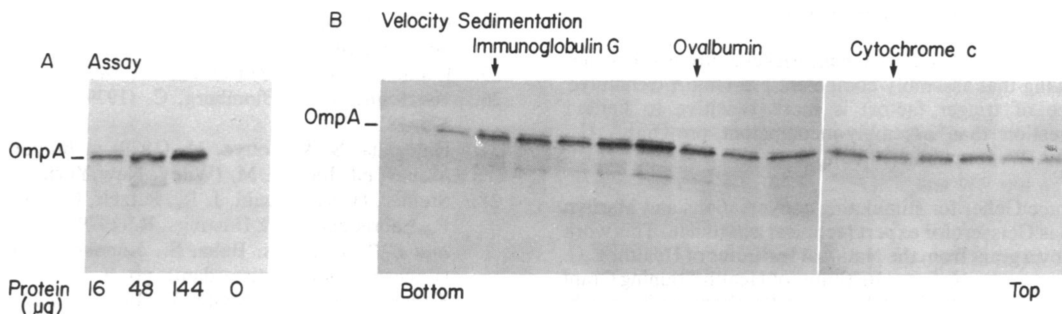

FIG. 6. Characterization of the trigger factor. Trigger factor-dependent [^{35}S]pro-OmpA was prepared as described for Fig. 4*A*. (*A*) Aliquots (80 μl) were mixed with 0, 16, 48, and 144 μg of S100 protein and dialyzed as described for Fig. 2. Equal quantities of [^{35}S]pro-OmpA were incubated with ATP, NADH, and membranes. Translocation was assayed by digestion with proteinase K. (*B*) S100 (6 mg of protein in 300 μl) was applied to a 15–30% glycerol gradient in buffer B and centrifuged as described for Fig. 5. Fractions (225 μl) were collected and protein content was determined. Protein from each fraction (16 μg) was added to 80-μl aliquots of trigger factor-dependent pro-OmpA and dialyzed as described for Fig. 2. Samples were incubated with ATP, NADH, and membranes and then incubated with proteinase K and analyzed by NaDodSO$_4$/PAGE and fluorography. Molecular weight marker proteins were analyzed as described for Fig. 5.

action and to genetically test its *in vivo* role in protein folding and translocation.

Previous studies have indicated that precursor proteins have different conformations from their mature membrane-bound or secreted forms and that these conformations are important for protein localization. *E. coli* leader peptidase was shown to be in a relatively protease-sensitive form prior to membrane assembly (22). Pre-maltose-binding protein is also initially in a protease-sensitive form; if it folds into the mature, protease-resistant conformation, it can no longer translocate across the plasma membrane (23). Eilers and Schatz (24) showed that fusion proteins comprising a mitochondrial cytochrome oxidase leader peptide joined to dihydrofolate reductase must unfold prior to their transit into the inner mitochondrial compartments.

It is not clear whether soluble proteins that are involved in protein translocation in eukaryotic organelles may, like the trigger factor, have a role in stabilizing or altering protein conformation. Mitochondrial protein uptake has been reported to involve a soluble, M_r 30,000 protein (25, 26) or a factor that contains an essential RNA (27). Signal-recognition particle is required for most protein translocation into the rough endoplasmic reticulum (3). Recent studies have shown that this factor can act late in the synthesis of a pre-secretory protein (28) or even posttranslationally (29). It too could have a role in influencing the folding pathway.

A fundamental problem in protein localization is how a protein might first fold in the aqueous environment of the cytoplasm, then pass through the hydrophobic core of a membrane, and finally emerge into the aqueous environment on the opposite membrane face. The signal hypothesis (30) proposed that proteins were extruded through the membrane as they emerge from the ribosome, thereby bypassing any folding until they reached the opposite membrane surface. However, protein translocation in mitochondria (31), chloroplasts (32), or bacteria (17, 33–35) can occur after translation is complete. Posttranslational translocation, or interaction with a membrane late in the growth of the polypeptide chain, has been reported for a number of proteins of the endoplasmic reticulum as well (see ref. 2 for a recent review). As suggested in the membrane trigger hypothesis (19), direct transfer model (36), loop models (37, 38), and helical hairpin hypothesis (39), conformation and the energetics of protein transfer between these phases may have a direct role in governing protein translocation. However, protein synthesis is slow, occurring on a time scale of seconds or minutes, whereas protein domains fold in much shorter times. Thus protein folding is likely to be a cotranslational event, and proteins that alter folding may have to interact cotranslationally. Factors such as the trigger factor reported here may stabilize intermediates or catalyze the correct folding of proteins prior to their membrane insertion.

Note Added in Proof. A folding function for trigger factor is supported by studies showing that assembly-competent pro-OmpA (renatured in the presence of trigger factor) is more sensitive to limited proteolytic digestion than assembly-incompetent pro-OmpA (L. Brundage and W. W., unpublished data).

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1. Wickner, W. & Lodish, H. F. (1985) *Science* **230**, 400–407.
2. Zimmermann, R. & Meyer, D. (1986) *Trends Biochem. Sci.* **11**, 512–515.
3. Walter, P. & Blobel, G. (1981) *J. Cell Biol.* **91**, 557–561.
4. Michaelis, S. & Beckwith, J. (1982) *Annu. Rev. Microbiol.* **36**, 435–465.
5. Muller, M. & Blobel, G. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7421–7425.
6. Chen, L., Rhoads, D. & Tai, P. C. (1985) *J. Bacteriol.* **161**, 973–980.
7. Chen, L. & Tai, P. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4384–4388.
8. Geller, B. L., Movva, R. & Wickner, W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4219–4222.
9. Muller, M. & Blobel, G. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7737–7741.
10. Rhoads, D. B., Tai, P. C. & Davis, B. D. (1984) *J. Bacteriol.* **159**, 63–70.
11. Gold, L. M. & Schweiger, M. (1971) *Methods Enzymol.* **20**, 537–542.
12. Bacallao, R., Crooke, E., Shiba, K., Wickner, W. & Ito, K. (1986) *J. Biol. Chem.* **261**, 12907–12910.
13. Campbell, D. H., Garvey, J. S., Cremer, N. E. & Sussdorf, D. H. (1970) *Methods in Immunology* (Benjamin, London).
14. Marchalonis, J. J. (1969) *Biochem. J.* **113**, 299–305.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–270.
16. Ito, K., Date, T. & Wickner, W. (1980) *J. Biol. Chem.* **255**, 2123–2130.
17. Zimmermann, R. & Wickner, W. (1983) *J. Biol. Chem.* **258**, 3920–3925.
18. Pigiet, V. P. & Schuster, B. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7643–7647.
19. Wickner, W. (1979) *Annu. Rev. Biochem.* **48**, 23–45.
20. Chen, R., Schmidmayr, W., Kramer, C., Chen-Schmeisser, U. & Henning, U. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4592–4596.
21. Oliver, D. B. & Beckwith, J. (1982) *Cell* **30**, 311–319.
22. Wolfe, P. B. & Wickner, W. (1984) *Cell* **36**, 1067–1072.
23. Randall, L. L. & Hardy, S. J. S. (1986) *Cell* **46**, 921–928.
24. Eilers, M. & Schatz, G. (1986) *Nature (London)* **322**, 228–232.
25. Miura, S., Mori, M. & Tatibana, M. (1983) *J. Biol. Chem.* **258**, 6671–6674.
26. Ohta, S. & Schatz, G. (1984) *EMBO J.* **3**, 651–657.
27. Firgaira, F. A., Hendrick, J. P., Kalousek, F., Kraus, J. P. & Rosenberg, L. E. (1984) *Science* **226**, 1319–1322.
28. Ainger, K. J. & Meyer, D. I. (1986) *EMBO J.* **5**, 951–955.
29. Mueckler, M. & Lodish, H. (1986) *Nature (London)* **322**, 549–552.
30. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 852–862.
31. Hallermayer, G. & Neupert, W. (1976) in *Genetics and Biogenesis of Chloroplasts and Mitochondria*, ed. Buchler, T. (Elsevier/North Holland, Amsterdam), pp. 813–818.
32. Grossman, A., Bartlett, S. & Chua, N. H. (1980) *Nature (London)* **285**, 625–628.
33. Ito, K., Sato, T. & Yura, T. (1977) *Cell* **11**, 551–559.
34. Ito, K., Mandel, G. & Wickner, W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1199–1203.
35. Randall, L. L. (1983) *Cell* **33**, 231–240.
36. vonHeijne, G. & Blomberg, C. (1979) *Eur. J. Biochem.* **97**, 175–181.
37. Halegoua, S. & Inouye, M. (1979) in *Bacterial Outer Membranes*, ed. Inouye, M. (Wiley, New York), pp. 67–114.
38. Steiner, D. F., Duguid, J. R., Patzelt, C., Chan, S. J., Quinn, P., Labrecque, A. & Hastings, R. (1979) in *Proinsulin, Insulin, and C-Peptide*, eds. Baba, S., Kaneko, T. & Yanaihara, N. (Excerpta Med., Amsterdam), pp. 9–19.
39. Engelman, D. M. & Steitz, T. (1981) *Cell* **23**, 411–422.