The EMBO Journal vol.8 no.3 pp.961 - 966, 1989

SecA protein hydrolyzes ATP and is an essential component of the protein translocation ATPase of *Escherichia coli*

Roland Lill, Kyle Cunningham, Lorna A.Brundage, Koreaki Ito[1], Donald Oliver[2] and William Wickner[1]

Molecular Biology Institute and Department of Biological Chemistry, University of California, 405 Hilgard Avenue, Los Angeles, CA 90024-1570, USA, [1]Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto, Japan, and [2]Department of Microbiology, State University of New York at Stony Brook, Stony Brook, NY 11794, USA

Communicated by W.Neupert

Bacterial protein export requires two forms of energy input, ATP and the membrane electrochemical potential. Using an *in vitro* reaction reconstituted with purified soluble and peripheral membrane components, we can now directly measure the translocation-coupled hydrolysis of ATP. This translocation ATPase requires inner membrane vesicles, SecA protein and translocation-competent proOmpA. The stimulatory activity of membrane vesicles can be blocked by either antibody to the SecY protein or by preparing the membranes from a *sec*Y-thermosensitive strain which had been incubated at the non-permissive temperature *in vivo*. The SecA protein itself has more than one ATP binding site. 8-azido-ATP inactivates SecA for proOmpA translocation and for translocation ATPase, yet does not inhibit a low level of ATP hydrolysis inherent in the isolated SecA protein. These data show that the SecA protein has a central role in coupling the hydrolysis of ATP to the transfer of pre-secretory proteins across the membrane.

Key words: membrane assembly/trigger factor/photo-inactivation

Introduction

Protein transfer from the cytoplasm to non-cytoplasmic compartments requires metabolic energy. ATP is required for the assembly of proteins into eukaryotic endoplasmic reticulum (Hansen *et al.*, 1986; Rothblatt and Meyer, 1986; Waters and Blobel, 1986; Wiech *et al.*, 1987), mitochondria (Pfanner and Neupert, 1986; Eilers *et al.*, 1987), chloroplasts (Grossman *et al.*, 1980; Flugge and Hinz, 1986), peroxisomes (Imanaka *et al.*, 1987) and nuclei (Newmeyer *et al.*, 1986), as well as in protein export to the cell surface of *Escherichia coli* (Chen and Tai, 1985). One role of ATP may be to act in concert with cytoplasmic proteins in order to maintain conformations that are essential for translocation (Rothman and Kornberg, 1986). Substantial evidence has accumulated for important roles of ATP-binding heat shock proteins in endoplasmic reticulum and mitochondrial import (Deshaies *et al.*, 1988; Chirico *et al.*, 1988). Moreover, later steps of mitochondrial import have also been found to require ATP (Pfanner *et al.*, 1987).

The efficient export of bacterial proteins across the plasma membrane has been shown to require both the membrane electrochemical potential and ATP (Geller *et al.*, 1986). However, the role of each energy source in translocation has remained elusive. We have found that *in vitro* translocation reconstituted with isolated membranes, purified trigger factor and proOmpA, still requires both energy sources for maximum efficiency (Crooke *et al.*, 1988a). Chen and Tai (1987) have shown that *E.coli* plasma membrane vesicles can be photo-inactivated for translocation with azido-ATP, suggesting that ATP is coupled to protein translocation by a membrane protein. We now report conditions for the direct assay of the protein translocation ATPase. This ATPase activity requires proOmpA (or, presumably, other presecretory proteins), the SecY/PrlA protein, and the SecA protein, a peripheral membrane protein (Oliver and Beckwith, 1982) which is essential for *in vitro* protein translocation (Cabelli *et al.*, 1988; Cunningham *et al.*, 1989). The SecA protein has ATP-reactive sites which are sensitive to 8-azido-ATP and an 8-azido-ATP insensitive catalytic site which hydrolyzes ATP.

Results

Assay of the translocation ATPase

ATP hydrolysis is needed for the translocation of proOmpA and other exported protein precursors across the *E.coli* plasma membrane (Chen and Tai, 1985). To allow direct measurement of the translocation ATPase, we have purified proOmpA to homogeneity (Crooke *et al.*, 1988b), eliminating soluble ATPases. The ATPase activity associated with membranes was minimized by treating the membranes with 6 M urea. Though this procedure inactivated the vesicles for translocation, activity was restored by the addition of pure SecA protein (Cunningham *et al.*, 1989). A substantial ATP hydrolytic activity was seen in the presence of SecA protein, proOmpA and urea-treated *E.coli* inner membrane vesicles (Table I). Omission of any one of these components substantially reduced the ATPase activity. We refer to this activity as 'translocation ATPase'.

Measurement of this translocation ATPase is related to the membrane transport of proOmpA. If proOmpA is inactivated by dilution from urea and incubation at higher temperature (Crooke *et al.*, 1988a,b), it was relatively ineffective in stimulating the translocation ATPase (Figure 1). Furthermore, the concentrations of competent proOmpA which stimulate translocation ATPase to half maximal levels are comparable to the concentrations needed to saturate membranes for translocation of proOmpA (Crooke *et al.* 1988b).

SecY/PrlA protein is a component of the translocation ATPase

SecY/PrlA has previously been shown to be essential for the *in vitro* translocation of proOmpA into inverted, sealed plasma membrane vesicles of *E.coli* (Bacallao *et al.*, 1986;

Table I. SecA protein, proOmpA and inner membrane vesicles are required for maximal translocation ATPase activity

Reaction No.	Inner membranes	SecA	ATPase activity (units/10 μl) −proOmpA	+proOmpA
1	−	−	5	1
2	−	+	20	18
3	+	−	21	19
4	+	+	60	294

Each reaction contained [γ-^{32}P]ATP (1 mM; 13−24 c.p.m./pmol) and (where indicated) SecA (56 μg/ml) in buffer B. ProOmpA (2 mg/ml) in buffer U was diluted 50-fold into each reaction (where indicated) and immediately warmed to 40°C to initiate the reaction. Aliquots were removed at time intervals and the initial rates of ATP hydrolysis were determined for each reaction within the first 12 min of incubation. All membranes were washed with 6 M urea (Cunningham *et al.*, 1989) and added to 0.1 mg/ml where indicated. Membranes from strain KM9 (*unc*$^-$) were employed. KM9 is isogenic to D10 (unc$^+$) except that it lacks the F_1F_0 ATPase.

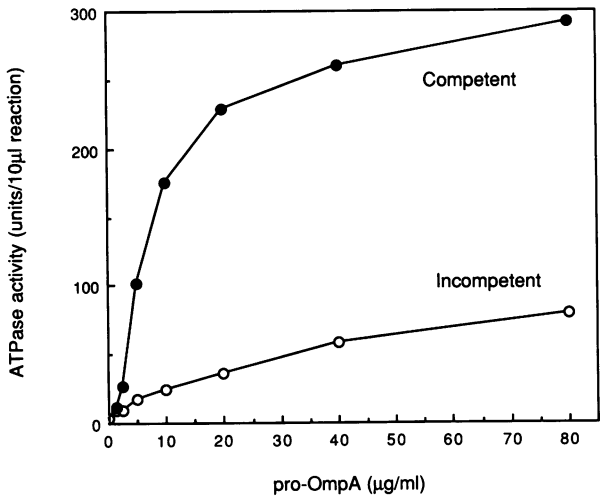

Fig. 1. Stimulation of SecA ATPase by competent and incompetent forms of proOmpA. Filled symbols: proOmpA in buffer U was diluted 25-fold into translocation reactions containing urea-washed KM9 inner membranes (100 μg/ml), SecA protein (50 μg/ml), [γ-^{32}P]ATP (1 mM; 10 c.p.m./pmol) in buffer B plus 50 mg/ml BSA. The mixtures were analyzed for translocation ATPase activity as described in Materials and methods. Open symbols: to obtain the translocation-incompetent form of proOmpA, aliquots of proOmpA in buffer U were diluted 20-fold into buffer B plus 50 mg/ml BSA and incubated at 40°C for 1 h. The samples were then mixed with inner membranes, SecA protein and ATP as described above and analyzed for translocation ATPase activity.

Fandl and Tai, 1987). Akiyama and Ito (1987) noted that the membrane-bound SecY/PrlA protein is sensitive to *in situ* proteolysis; other membrane proteins may be required as well for full activity of the translocation ATPase. Membranes lose their stimulatory activity for ATP hydrolysis when they are treated with protease (Table II). Membranes derived from SecY-thermosensitive strain which had been incubated at the nonpermissive temperature *in vivo* lack the stimulatory activity for the translocation ATPase (Table II, line 6). A more direct test of SecY function in the ATPase reaction is to use a specific inhibitor. An antibody was raised against a synthetic peptide derived from the N-terminus of the SecY/PrlA sequence (Cerretti *et al.*, 1983); as previously shown (M.Watanabe and G.Blobel, personal communication), such antibody inhibits the overall translocation

Table II. SecY protein is required for translocation ATPase

Reaction No.	Inner membranes	ATPase activity (units/10 μl) −proOmpA	+proOmpA
1	none	16	16
2	KM9	21	243
3	KM9:trypsin	4	10
4	KM9 + KM9:trypsin	7	217
5	D10	29	260
6	CJ107	19	28
7	D10 + CJ107	32	216

ATPase assays were performed as described in Table I. For the experiment in line 3, KM9 membranes were diluted to 0.5 mg/ml in buffer B and treated with 0.1 mg/ml trypsin at 0°C for 30 min. The protease was then inhibited with phenylmethylsulfonylfluoride (25 μl of a fresh saturated solution in buffer B per 100 μl of membrane suspension) and soybean trypsin inhibitor (0.2 mg/ml suspension). Membranes were re-isolated by centrifugation (150 000 *g*, 15 min, 4°C, in a Beckman airfuge) before use. For the experiment in line 4, protease-treated KM9 membranes were mixed with an equivalent amount of untreated membranes prior to addition to the assay. For the experiment in lines 6 and 7, membranes were prepared from strain CJ107 (unc$^+$, secY24-ts) which had been grown at 42°C for 2 h to express the secY mutant phenotype.

Table III. Translocation ATPase is inhibited by antibodies to SecY protein

Reaction	SecY-IgG	Control IgG	SecY peptide	Translocation ATPase activity
1	−	−	−	278
2	−	−	+	278
3	+	−	−	17
4	+	−	+	246
5	−	+	−	213

Where indicated, urea washed KM9 membranes (120 μg/ml) were incubated in the presence of anti-SecY IgG (2.5 mg/ml) and/or synthetic SecY(2−22) peptide (25 μM) for 3 h at 0°C prior to the ATPase assay. SecA protein (50 μg/ml) and [γ-^{32}P]ATP (1 mM; 10 c.p.m./pmol) were then added to each suspension and the initial ATPase activity was determined as the difference between the activities in the presence of added proOmpA (80 μg/ml) and its absence, as described in Materials and methods. To prepare antiserum to the SecY/PrlA protein, a synthetic peptide corresponding to residues 2−22 of the amino-terminus of the protein (Cerretti et al., 1983), with an additional carboxy-terminal cysteine, was coupled to keyhole limpet hemocyanin (Green et al., 1982) and injected into rabbits.

reaction (data not shown). Antibody to the SecY/PrlA protein completely prevented membranes from stimulating the translocation ATPase (Table III), while control immunoglobulin had no such effect. The peptide antigen itself had no effect on the translocation ATPase, but it relieved the inhibition by the antibody to SecY/PrlA protein. While our data do not yet permit us to determine whether the SecY/PrlA protein stimulates the translocation ATPase by direct interaction, this would be the simplest basis for our results.

The role of SecA in translocation ATPase

To establish that the translocation ATPase requires the SecA protein itself and not a contaminant in the preparation, we assayed the ability of fractions from the gel filtration step of SecA protein purification to support the translocation ATPase (Figure 2). The translocation ATPase activity (filled squares), assayed in the presence of proOmpA and mem-

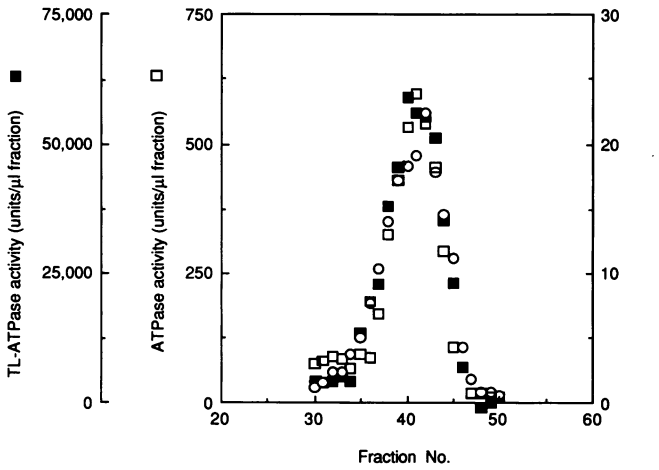

Fig. 2. Co-purification of SecA protein, SecA ATPase activity, and translocation ATPase activity. A SecA protein preparation which had been purified by chromatography on phosphocellulose and ammonium sulfate precipitation (Cunningham *et al.*, 1989) was applied to a Sephacryl S-300 column in buffer Q (50 mM Tris−Cl, pH 7.6, 10% glycerol, 1 mM dithiothreitol) at 4°C. Fractions were collected and analyzed for total protein by the Bradford method (○) and for ATPase activity. SecA ATPase was measured using 0.24 μl of each fraction without additional membranes or proOmpA (□). Translocation ATPase was measured (■) using 0.006 μl of each fraction, 80 μg/ml urea-treated KM9 membranes and 67 μg/ml proOmpA in a 30 μl reaction.

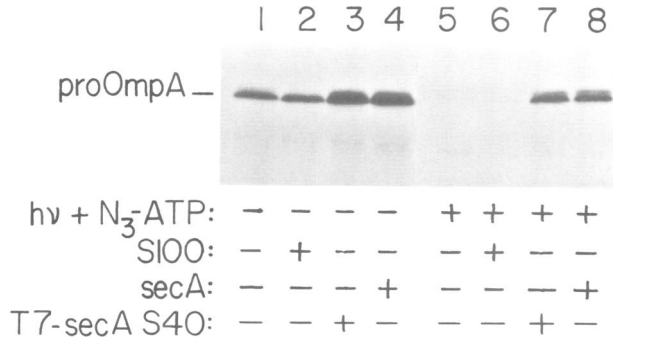

Fig. 3. Translocation of proOmpA into azido-ATP inactivated inner membrane vesicles is restored by purified SecA protein. Inner membrane vesicles of *E.coli* strain D10 were UV-irradiated for 15 min on ice in the presence of 15 mM $8N_3$-ATP in 20 μl of buffer X. Translocation reactions with [^{35}S]proOmpA diluted from 8 M urea were then performed as detailed in Materials and methods using 2.2 μg of either untreated membranes (**lanes 1−4**) or irradiated vesicles (**lanes 5−8**). Samples 2 and 6 contained 17 μg of S100 protein of strain D10, sample 3 and 7 had 12 μg of S40 protein of the SecA-overproducer strain BL21 (λDE3)/pT7-secA (Cabelli *et al.*, 1988), and lanes 4 and 8 contained 1 μg of purified SecA protein.

brane vesicles, was coincident with the protein profile (open circles), confirming that ATP hydrolysis requires the SecA protein. We observed that the low level of ATPase activity of SecA protein in the absence of added membranes or proOmpA (Table I) is also coincident with the SecA protein profile on gel filtration (Figure 2, open squares), and therefore due to the SecA protein rather than a contaminant. We refer to this activity as the 'SecA ATPase'.

To explore the role of the SecA ATPase in the translocation ATPase activity, we employed the photoreactive ATP analog 8-azido-ATP. As previously reported (Chen and Tai, 1987), *E.coli* plasma membrane vesicles can be inactivated for translocation by UV-irradiation with 8-azido-ATP (Figure 3, lanes 1 versus 5). The azido-ATP inactivated membranes can be re-activated for translocation by addition of an extract from a strain which overproduces the SecA protein (lane 7) or (lane 8) by purified SecA protein (Cabelli *et al.*, 1988; Cunningham *et al.*, 1989). A cytoplasmic fraction from wild-type *E.coli*, termed S100, provides no stimulation of translocation (lane 6) at the concentrations tested, presumably because it contains insufficient SecA protein. We also observed that the SecA protein is selectively released from 8-azido-ATP derivatized membranes, and that 8-azido-ATP derivatized SecA will not bind to membranes (R.Lill, unpublished). Thus it is likely that SecA is the azido-ATP sensitive component of the membrane.

To establish the relationship between the azido-ATP sensitivity of translocation, the translocation ATPase and the SecA ATPase, purified SecA protein was derivatized by 8-azido[α-^{32}P]ATP (Figure 4). Quantitation of the extent of labeling yielded 2 mol of azido-ATP per mol of protein (Figure 4B). This figure is based on quantitative amino acid analysis, which indicated that the true SecA protein concentration is 60% of that determined by the methods of Bradford (1976) or Lowry *et al.* (1951) using bovine serum albumin as a standard. The derivatized SecA protein is no longer capable of supporting translocation into inner membrane vesicles (Figure 4C) which, by urea treatment (Cunningham *et al.*, 1989), had been made dependent on added SecA protein. The concentration dependence of inactivation of SecA protein for translocation parallels its derivatization. Both derivatization of the protein and its inactivation for supporting proOmpA translocation were blocked by the inclusion of γS-ATP during the photolysis with 8-azido-ATP. Derivatization also inactivated SecA protein for its participation in the translocation ATPase (Figure 4D). However, the azido-ATP derivatization of the SecA protein actually caused a slight increase in SecA ATPase activity (Figure 4E). These data indicate that the SecA protein has three sites which are reactive with azido-ATP and ATP. One site hydrolyzes ATP but is insensitive to derivatization by 8-azido-ATP. We do not have direct evidence of whether this hydrolytic site is directly involved in translocation, though this is an attractive possibility. The other sites on the SecA protein can be derivatized by 8-azido-ATP and are needed for protein translocation and translocation ATPase. The separate functions of each of these ATP binding sites remains to be determined. One tool for this may be 2-azido-ATP (Czarnecki, 1984) which completely inactivates translocation ATPase and at least partially inactivates the SecA ATPase activity (R.Lill, unpublished).

Discussion

The SecA protein reversibly binds to the plasma membrane, where it interacts with proOmpA to support translocation of proOmpA and the hydrolysis of ATP. At least one membrane element which is essential for the translocation ATPase is sensitive to proteolysis, is missing or inactive in membranes from a *sec*Y-thermosensitive strain which had been incubated at the non-permissive temperature, and is inactivated when antibody is bound to the SecY protein. These oservations add considerable detail to our working model (Lill *et al.*, 1988) of the translocation of proOmpA (and, we presume, other pre-secretory and membrane proteins) across the plasma membrane. In this model (Figure

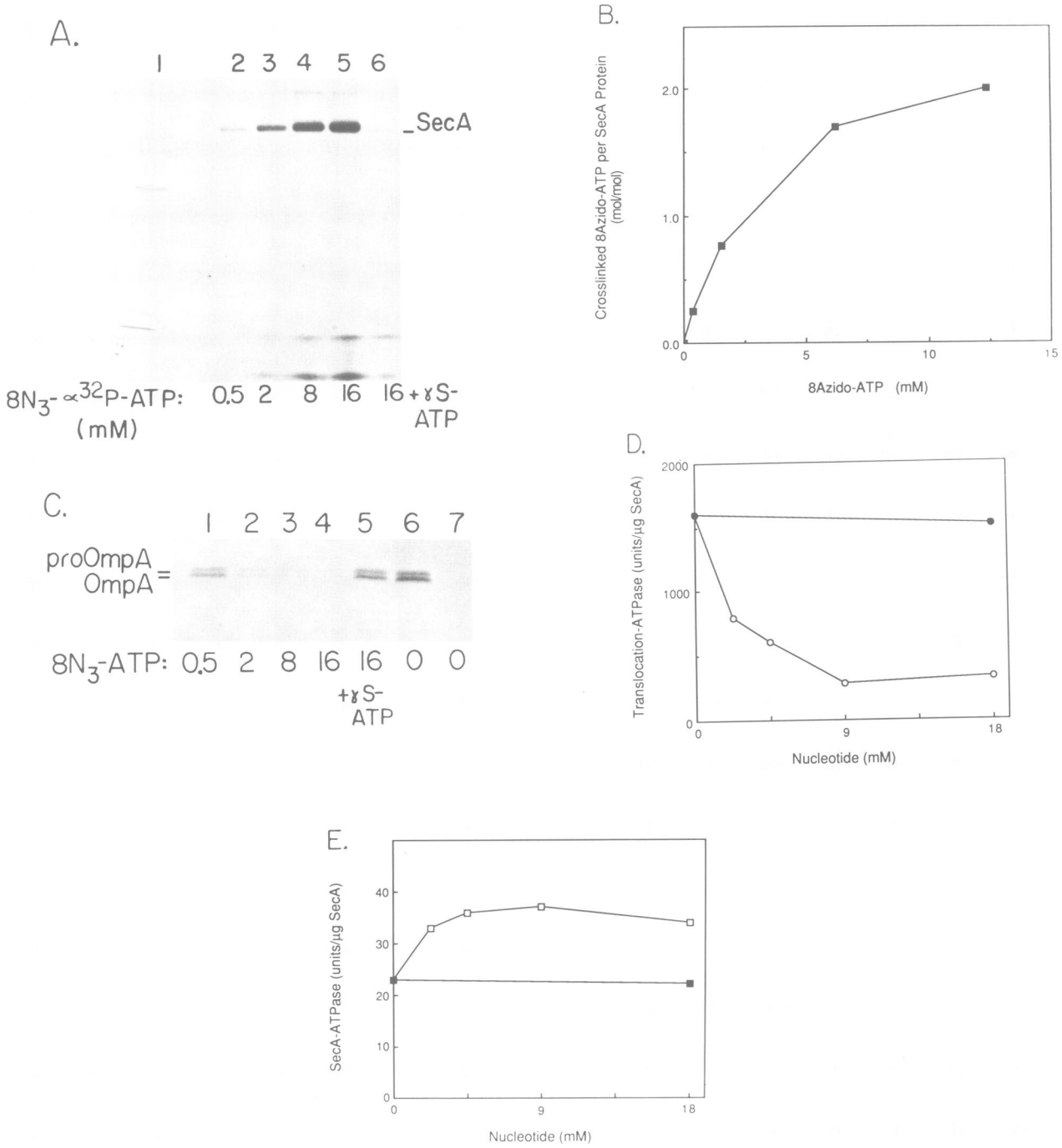

Fig. 4. Inactivation of SecA protein by photo-crosslink with azido-ATP. Samples of SecA protein (100 μg) were UV-irradiated with the indicated amounts of $8N_3$-[α-^{32}P]ATP (21 c.p.m./pmol) in 100 μl of buffer X for 15 min on ice as described in Materials and methods. An equimolar concentration of γS-ATP (16 mM) was added to the sample shown in lane 7 of parts A and C prior to irradiation. Protein was precipitated with ammonium sulfate (0.5 g/ml) in buffer A (50 mM Hepes–KOH, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol) containing 10 mM ATP for 14 h at 0°C and pelleted by ultracentrifugation (Beckman SW50.1 rotor, 40 000 r.p.m., 30 min, 2°C). The pellet was suspended three times in 0.6 ml of this ammonium sulfate/ATP solution and collected by ultracentrifugation, then finally dissolved in 100 μl of buffer A. Protein recovery was assayed by the method of Bradford (1976). (**A**) Samples of irradiated SecA protein (2.5 μg) were analyzed by SDS–PAGE and autoradiography. Lane 1 contains molecular weight markers. (**B**) To quantify the stoichiometry with which SecA protein was labeled by 8-azido-ATP, residual free nucleotide (see panel A) was removed by gel filtration. SecA protein (30 μg) in 100 μl of buffer A containing 10 mM ATP was applied to a Sephadex G25 column (1.4 × 6.5 cm, 10 ml/cm^2·h flow rate, 4°C). The void volume fractions were assayed for protein and radioactivity to allow calculation of stoichiometry. (**C**) Translocation reactions were performed using 5.5 μg of urea-washed inner membrane vesicles, isolated from strain KM9 (Cunningham *et al.*, 1989) and 0.5 μg of irradiated SecA protein. The sample in lane 6 contained 0.5 μg of untreated SecA protein, while the sample in lane 7 had no SecA protein. For measuring the translocation ATPase (**D**) and the SecA ATPase (**E**), the irradiations were performed using non-radioactive $8N_3$-ATP as indicated. After precipitation with ammonium sulfate (0.5 g/ml) in buffer A and sedimentation as above, the pellet was suspended in buffer A without further washes. The translocation ATPase and SecA ATPase were measured as described in Figure 2 using 9 and 300 μg/ml, respectively, of irradiated SecA protein (open symbols) or of SecA protein irradiated with the indicated concentrations of ATP, as a control (filled symbols).

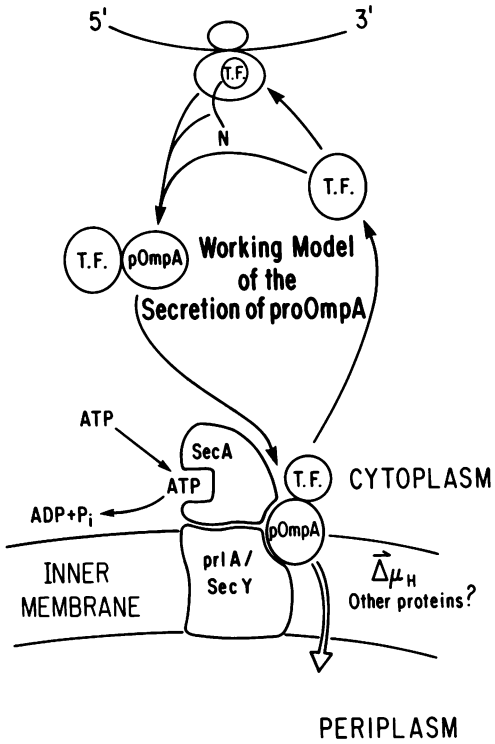

Fig. 5. A working model of the secretion of proOmpA across the plasma membrane of *E.coli*. See text for details. Trigger factor is designated as T.F.

5), proOmpA forms a 1:1 complex with trigger factor either during, or shortly after, its synthesis (Crooke *et al.*, 1988b). Trigger factor is an abundant protein of *E.coli* which is found both in the cytoplasm and bound to the 50S ribosomal subunit. *In vitro*, trigger factor stabilizes proOmpA for membrane assembly. The trigger factor–proOmpA complex then binds to membranes, which releases the trigger factor (S.Lecker and W.Wickner, unpublished). Trigger factor is presumably recycled to the cytoplasm and can rebind to the ribosomes (Lill *et al.*, 1988). ProOmpA then interacts with SecA to stimulate ATP hydrolysis, and the proOmpA translocates across the membrane. We have not determined whether the SecA ATPase active site is also the hydrolytic site for the translocation ATPase; however, this is the simplest working hypothesis. The SecA protein must be present on the membrane prior to proOmpA binding to result in subsequent translocation (Cunningham *et al.*, 1989). The SecY/PrlA protein is required for proOmpA translocation and for proOmpA-dependent ATP hydrolysis by SecA protein. Our working hypothesis (Figure 5) is that this reflects a direct, physical interaction of these three proteins. The 8-azido-ATP sensitive sites on the SecA protein may control interaction between the SecA and SecY proteins. However, it should be emphasized that the functional interactions between SecA protein, SecY/PrlA protein and proOmpA could also require other integral membrane proteins.

It is not yet clear how the translocation ATPase couples the hydrolysis of ATP to the translocation process. Under our assay conditions, ~1000 molecules of ATP are hydrolyzed per added proOmpA molecule. This might be due to unsealed membranes in our preparations, to the lack of a membrane potential under translocation ATPase assay conditions, or to the absence of another factor(s). It will be important to seek the factors, or conditions, which more closely couple ATP hydrolysis with translocation. While SecY protein and competent proOmpA are required for both membrane assembly and for the ATPase activity, and the concentrations of SecA protein needed for these two activities are comparable (K.Cunningham and R.Lill, unpublished observations), further studies with the purified components are needed to establish the direct binding relationships proposed in Figure 5.

ATP hydrolysis is coupled to protein translocation in essentially all translocation-competent membranes which have been examined. This includes bacteria, chloroplasts, mitochondria, nuclei and endoplasmic reticulum. To the degree that the mechanisms of translocation are highly conserved, the ATP hydrolysis of protein translocation into other organelles might also be coupled to the presence of the translocating protein. It remains to be determined whether the functions supported by the hydrolysis of ATP are conserved as well.

Materials and methods

Materials

ProOmpA was isolated as described in Crooke *et al.* (1988b), then precipitated with trichloroacetic acid, suspended twice in ice-cold acetone to remove the acid, and finally dissolved in buffer U (8 M urea, 50 mM Tris–Cl, pH 8.0, 1 mM dithiothreitol). SecA protein employed in all experiments was isolated as described (Cunningham *et al.*, 1989). TPCK-trypsin was from Boehringer-Mannheim. Unless otherwise noted, protein was assayed by the methods of Lowry *et al.* (1951) and Bradford (1976) with bovine serum albumin (BSA) as a standard. Other materials and methods are as described in the accompanying article (Cunningham *et al.*, 1989).

Assay of ATP hydrolysis

The release of radioactive orthophosphate from [γ-^{32}P]ATP (ICN radiochemicals) was monitored by the procedure of Sander *et al.* (1975), with minor modifications. Briefly, reactions were performed in buffer B (50 mM Hepes–KOH, ph 7.5, 50 mM KCl, 5 mM $MgCl_2$) with [γ-^{32}P]ATP (1 mM, 10–50 c.p.m./pmol), urea-treated KM9 inner membrane vesicles (100 μg/ml; Cunningham *et al.*, 1989), SecA protein (50 μg/ml) and proOmpA (80 μg/ml). Reactions (25 μl final volume) were mixed at 0°C and incubated at 40°C immediately after addition of proOmpA in buffer U (8 M urea, 50 mM Tris–Cl, pH 8.0, 1 mM dithiothreitol). The reaction was terminated after 10 min by addition of 175 μl of 1 M perchloric acid, 1 mM sodium phosphate. Radioactivity was determined by Cerenkov counting. Ammonium molybdate (0.4 ml of 20 mM) and 0.4 ml of isopropyl acetate were added to each tube. The tubes were vortexed vigorously for 5 s and centrifuged for 10 s in a Brinkman microfuge to separate the organic and aqueous phases. A portion of the top (organic) phase containing the radioactive orthophosphate–molydate complex was removed and assayed for radioactivity. One unit of ATPase activity produced 1 pmol of orthophosphate per min under these conditions. Due to the relatively large amount of ATP hydrolysis associated with inner membranes even in the absence of added SecA protein or proOmpA, all assays for translocation ATPase were performed with, and without, addition of proOmpA; the difference between these two activities represents 'translocation' ATPase activity.

Irradiations with azido-ATP

Photo-crosslinking was performed with $8N_3$-ATP (Sigma, St Louis, MO) or with the α-^{32}P-labeled nucleotide (ICN, Irvine). The concentration of nucleotide was calculated using the extinction coefficient of 1.13×10^4 M^{-1} cm^{-1} (Haley and Hoffman, 1974). Unless stated otherwise, 5–30 μg of inner membranes or 300 ng of purified SecA protein were mixed with 15 mM of the photo-crosslinking reagent in 20–40 μl of buffer X [50 mM Hepes–KOH, pH 7.5, 10 mM $Mg(OAc_2)$] at 0°C under reduced light. The mixtures were placed onto the wells of a microscope slide fixed in a Petri dish on ice. They were irradiated with the 366 nm wavelength band of a UV handlamp UV GL-25 (UV Products Inc., San Gabriel) for 15 min on ice from 3 cm distance. Samples were then analyzed by the proOmpA translocation assay or by SDS–PAGE and fluorography as detailed in the text.

Acknowledgements

We thank Marilyn Rice and Douglas Geissert for expert technical assistance and Zhixiong Xue (UCLA) for advice on the synthesis and use of azido-ATP compounds. This work was supported by a grant from the NIH. R.L. is a fellow of the Deutsche Forschungsgemeinschaft. L.A.B. is a predoctoral trainee supported by USPHS National Institutional Research Service Award CA-09056.

References

Akiyama,Y. and Ito,K. (1987) *EMBO J.* **6**, 3465–3470.
Bacallao,R., Crooke,E., Shiba,K., Wickner,W. and Ito,K. (1986) *J. Biol. Chem.* **261**, 12907–12910.
Bradford,M.M. (1976) *Anal. Biochem.* **72**, 248–254.
Cabelli,R., Chen,L.L., Tai,P.C. and Oliver,D.B. (1988) *Cell*, **55**, 683–692.
Cerretti,D.P., Dean,D., Davis,G.R., Bedwell,D.M. and Nomura,M. (1983) *Nucleic Acids Res.*, **11**, 2599–2615.
Chen,L. and Tai,P.C. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4384–4388.
Chen,L. and Tai,P.C. (1987) *Nature*, **328**, 164–166.
Chirico,W.J., Waters,M.G. and Blobel,G. (1988) *Nature*, **332**, 805–810.
Crooke,E., Brundage,L., Rice,M. and Wickner,W. (1988a) *EMBO J.*, **7**, 1831–1835.
Crooke,E., Guthrie,B., Lecker,S., Lill,R. and Wickner,W. (1988b) *Cell*, **54**, 1003–1011.
Cunningham,K., Lill,R., Crooke,E., Rice,M., Wickner,W. and Oliver,D. (1989) *EMBO J.*, **8**, 955–959.
Czarnecki,J.J. (1984) *Biochim. Biophys. Acta*, **800**, 41–51.
Deshaies,R.J., Koch,B.D., Werner-Washburne,M., Craig,E.A. and Schekman,R. (1988) *Nature*, **332**, 800–805.
Eilers,M., Oppliger,W. and Schatz,G. (1987) *EMBO J.*, **6**, 1073–1077.
Fandl,J.P. and Tai,P.C. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 7448–7452.
Flugge,U.I. and Hinz,G. (1986) *Eur. J. Biochem.*, **160**, 563–570.
Geller,B., Movva,N.R. and Wickner,W. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 4219–4222.
Green,N., Alexander,H., Olson,A., Alexander,S., Shinnick,T.M., Sutcliffe,J.G. and Lerner,R.A. (1982) *Cell*, **28**, 477–487.
Grossman,A., Bartlett,S., and Chua,N.-H. (1980) *Nature*, **285**, 625–628.
Haley,B.E. and Hoffman,J.F. (1974) *Proc. Natl. Acad. Sci. USA*, **71**, 3367–3371.
Hansen,W., Garcia,P.D. and Walter,P. (1986) *Cell*, **45**, 397–406.
Imanaka,T., Small,G.M. and Lazarow,P.B. (1987) *J. Cell Biol.*, **105**, 2915–2922.
Lill,R., Crooke,E., Guthrie,B. and Wickner,W. (1988) *Cell*, **54**, 1013–1018.
Lowry,O.H., Rosebrough,N.J., Farr,A.L. and Randall,R.J. (1951) *J. Biol. Chem.*, **193**, 265–270.
Newmeyer,D.D., Lucocq,J.M., Burglin,T.R. and DeRobertis,E.M. (1966) *EMBO J.*, **5**, 501–510.
Oliver,D.B. and Beckwith,J. (1982) *Cell*, **30**, 311–319.
Pfanner,N. and Neupert,W. (1986) *FEBS Lett.*, **209**, 152–156.
Pfanner,N., Tropschug,M. and Neupert,W. (1987) *Cell*, **49**, 815–823.
Rothblatt,J.A. and Meyer,D.I. (1986) *EMBO J.*, **5**, 1031–1036.
Rothman,J.E. and Kornberg,R.D. (1986) *Nature*, **322**, 209–210.
Sander,G., Marsh,R.C., Voigt,J. and Parmeggiani,A. (1975) *Biochemistry*, **14**, 1805–1814.
Waters,M.G. and Blobel,G. (1986) *J. Cell Biol.*, **102**, 1543–1550.
Wiech,H., Sagstetter,M., Muller,G. and Zimmermann,R. (1987) *EMBO J.*, **6**, 1011–1016.

Received on November 17, 1988; revised on December 29, 1988