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The SecY membrane component of the bacterial protein export machinery: analysis by new electrophoretic methods for integral membrane proteins

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The product of the *secY* (*prlA*) gene (the SecY protein) involved in protein export in *Escherichia coli* was overproduced and localized in the cytoplasmic (inner) membrane. Because of its strong interaction with a non-ionic detergent (NP40), it partitioned into the detergent layer during electroblotting through a NP40-containing gel (detergent blotting), and it formed a horizontal streak in the O'Farrell two-dimensional gel electrophoretic system. Consequently, we developed an alternative two-dimensional gel procedure, which proved useful for analysis of integral membrane proteins, especially in combination with detergent blotting. SDS-gel electrophoresis was carried out successively through gels of lower (first dimension) and higher (second dimension) sieving effects. Many membrane proteins, unlike soluble proteins, formed spots off and above the diagonal line, and all of these spots partitioned exclusively into the detergent layer. A characteristic pattern of integral membrane proteins of *E. coli* was thus obtained and the spot of the SecY protein in the cytoplasmic membrane was identified even when it was not overproduced. These results show that the gene *secY* specifies an integral membrane component of the protein export machinery.

Key words: Escherichia coli/membrane protein/protein secretion/two-dimensional gel electrophoresis

Introduction

Export of proteins through the cytoplasmic membrane in *Escherichia coli* may require, in addition to the signal sequence and other topogenic information carried within the polypeptide chain, the function of the 'protein export machinery'. Although little is known about the molecular nature of such machinery, genetic analyses indicate that genes *secA, secB,* and *secY (prlA)*, as well as *secC, prlB, prlC, prlD,* and *prlF* are involved in the process (Oliver and Beckwith, 1981; Kumamoto and Beckwith, 1983; Shiba *et al.*, 1984; Emr *et al.*, 1981; Ferro-Novick *et al.*, 1984; Bankaitis and Bassford, 1985; Kiino and Silhavy, 1984). The product of the *secA* gene has been identified as a 92 000-dalton protein which interacts weakly with the membrane (Oliver and Beckwith, 1982). Two enzymes that catalyze signal (leader) peptide cleavage have also been studied (Wolfe *et al.*, 1983; Yu *et al.*, 1984; Innis *et al.*, 1984).

The *secY (prlA)* gene is located within the *spc* ribosomal protein operon and is essential for protein export (Ito *et al.*, 1983; Shiba *et al.*, 1984). The product of *secY* is predicted from the DNA sequence of the gene to be a hydrophobic protein of 49 000 daltons (Cerretti *et al.*, 1983). We have previously identified the SecY protein using the maxicell system (Ito, 1984). The protein was found to share some unusual properties with membrane proteins such as the lactose carrier.

We have further characterized this protein and shown that it is a component of the cytoplasmic membrane. In these studies, we used some newly devised electrophoretic techniques. The detergent blotting procedure (K.Ito and Y.Akiyama, in preparation) demonstrated the amphiphilic nature of the SecY protein. Furthermore, a newly developed two-dimensional gel electrophoresis system was shown to be a powerful tool for analysis of integral membrane proteins including SecY.

Results

SecY protein is overproducible using a lac promoter vector

The gene product of *secY* was previously identified using the gene cloned in a plasmid under the control of the phage λ pL promoter. In this experiment, the maxicell system was essential for detecting the protein by SDS-gel electrophoresis, indicating the low amount of SecY synthesized. Moreover, the plasmids pNO1576 (Ito *et al.*, 1983), pKY3 and pKY6 (Shiba *et al.*, 1984), in which the *secY* gene was placed under *lac* promoter control and which complemented the *secY* mutation, did not give a visibly identifiable amount of the SecY protein (our unpublished results). We therefore examined pNO1573 (constructed, and kindly provided by M.Wittekind and M.Nomura), a derivative of pUC8 (Vieira and Messing, 1982) containing the 2.8-kb *Pst*I-*Pst*I fragment of the *spc* operon. This plasmid is very unstable (Wittekind and Nomura, personal communication). Certainly, in our hands, it could not be maintained even in a *lacI*^{q} (the *lac* repressor-overproducing) background in the presence of glucose. We found, however, that the plasmid was maintained stably in a *cya* (adenylate-cyclase deficient) derivative of the *lacI*^{q} strain.

Cells were induced by isopropyl-β-D-thiogalactoside (IPTG) and cyclic AMP, pulse-labeled with ^{35}S-methionine, and analyzed by SDS-gel electrophoresis followed by autoradiography. Cells carrying pNO1573 synthesized a large amount of the labeled SecY protein (Figure 1, lane 2). In addition, lower-molecular weight bands were visible. They presumably represent a β-galactosidase-S5 fusion protein and the L15 protein (L30 is also overproduced but not seen because of its low molecular weight). The SecY protein overproduced in this manner was unstable (data not shown) as was that studied previously in maxicells (Ito, 1984). The protein band assigned as SecY exhibited the same electrophoretic mobility as well as the same property of disappearing after heating in SDS at 100°C (Figure 1, lane 5) as the protein previously characterized in maxicells (Ito, 1984).

An inspection of the DNA sequences showed that the fusion joint between the N-terminal part of β-galactosidase and the DNA insert was in-frame in pNO1573, but was out-of-frame in plasmids pNO1576, pKY3 or pKY6. Thus, it is possible that SecY and the other *spc* operon proteins are synthesized at high rates in pNO1573 because the expression of these genes is translationally coupled to the upstream genes (Nomura *et al.*, 1984). This notion was supported by the following experiments. The *Pst*I fragment containing *secY* was isolated from pNO1573 and recloned into the *Pst*I site of pUC9 (Vieira and Messing, 1982), in which an out-of-frame joint would be generated between the

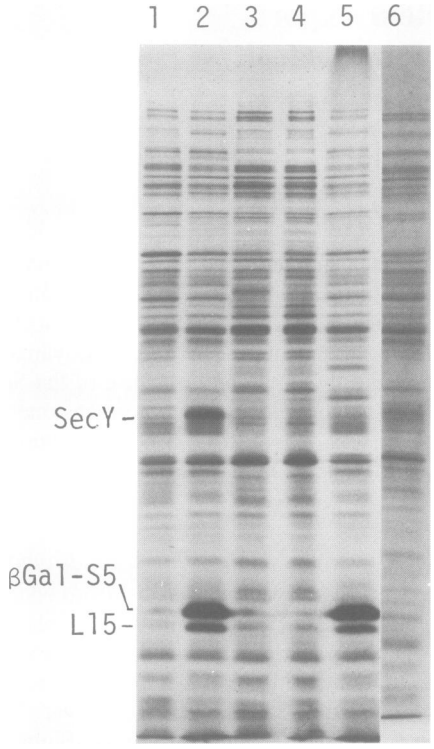

Fig. 1. Overproduction of the SecY protein. Cells of AD21/pNO1573 (**lanes 2, 4** and **5**), AD21/pUC8 (**lanes 1** and **3**), and AD21/pKY16 (**lane 6**) were induced (**lanes 1, 2, 5** and **6**) or uninduced (**lanes 3** and **4**) before labeling with [^{35}S]methionine. Whole-cell extracts were electrophoresed and proteins visualized by autoradiography. The sample for **lane 5** was heated at 100°C for 3 min before electrophoresis. βGal-S5 represents the putative fusion protein between the N-terminal part of β-galactosidase and the ribosomal S5 protein.

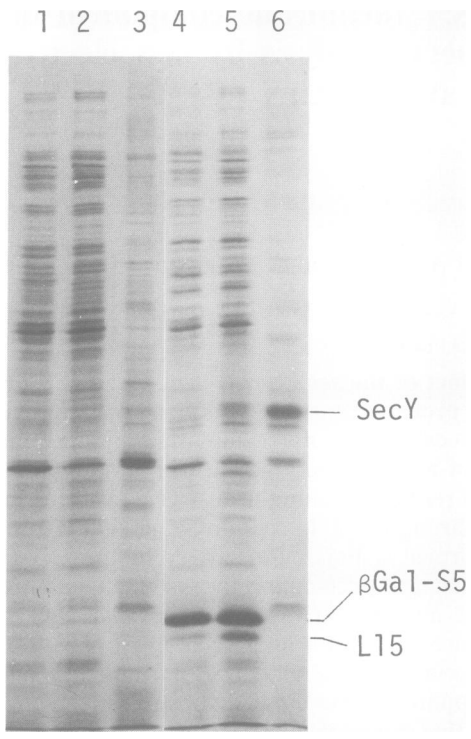

Fig. 2. Localization of the SecY protein. Induced cells of AD21/pUC8 (**lanes 1–3**) and AD21/pNO1573 (**lanes 4–6**) were labeled and fractionated into 'periplasmic' (**lanes 1** and **4**), cytoplasmic (**lanes 2** and **5**) and the membrane (**lanes 3** and **6**) fractions. Because some cell lysis occurred during the lysozyme-sucrose treatment with cells of this particular genetic background, the 'periplasmic fraction' was actually a mixture of periplasmic and cytoplasmic compartments.

lacZ and the *rpsE* (S5) genes. The resulting plasmid (pKY16) did not produce any identifiable amount of the SecY protein (Figure 1, lane 6). The same fragment, when reisolated from pKY16 and cloned back to pUC8, again produced a large amount of SecY. The copy number of pKY16 was no less than that of pNO1573, since the yield of plasmid DNA was consistently higher for pKY16 than pNO1573. Thus, the expression of *secY* appears to be dependent on the continued translation of the upstream genes.

SecY protein is located in the cytoplasmic membrane

The SecY protein was recovered from the rapidly-sedimenting membrane fraction (Figure 2, lane 6). Although a small amount of SecY was seen in the soluble cytoplasmic fraction (lane 5), it was probably due to contamination with small membrane fragments produced during sonication. Some cell lysis occurred with this particular *E. coli* strain during treatment by lysozyme and sucrose. Thus, the pattern of the 'periplasmic fraction' was almost identical to that of the cytoplasmic fraction, except that the former was devoid of the SecY protein (Figure 2, cf. lanes 4 and 5); a soluble fraction prepared by a mild cell lysis method does not contain the SecY protein. In an isopycnic sucrose gradient centrifugation of the membrane fraction, the SecY protein co-banded with the cytoplasmic (inner) membrane (Figure 3). Thus it cannot be in protein aggregates. From these results, we conclude that the SecY protein is a cytoplasmic membrane protein.

SecY protein is not tractable in the O'Farrell two-dimensional gel electrophoresis

In the next step, we wanted to identify the 'native' protein without overproduction. We first attempted to identify the protein spot by the established method of two-dimensional electrophoresis (O'Farrell *et al.*, 1977). Because the SecY protein was expected to be basic (Cerretti *et al.*, 1983), the non-equilibrium system was used. Samples were prepared with or without pretreatment with SDS (Ames and Nikaido, 1976). In either case, the overproduced SecY protein formed a horizontal streak while other proteins formed well-separated spots (data not shown). Thus, the two-dimensional technique of O'Farrell *et al.* (1977) cannot be applied to certain membrane components such as the SecY protein. Presumably, this is due to interaction between the protein and NP40, the non-ionic detergent being used for protein solubilization (see below).

SecY protein is amphiphilic as demonstrated by 'detergent blotting'

We recently developed a protein-blotting system (detergent blotting) for detecting integral membrane proteins separated by SDS-gel electrophoresis (Ito and Akiyama, in preparation). Briefly, a gel is subjected to electro-blotting through another gel containing a non-ionic detergent (NP40) onto a nylon membrane filter. Amphiphilic or integral membrane proteins partition into the detergent-containing gel whereas hydrophilic or soluble proteins are transferred through the detergent layer to the membrane filter. The overproduced SecY protein was trapped in the NP40-containing gel (see Figure 5), indicating that it interacts strongly with

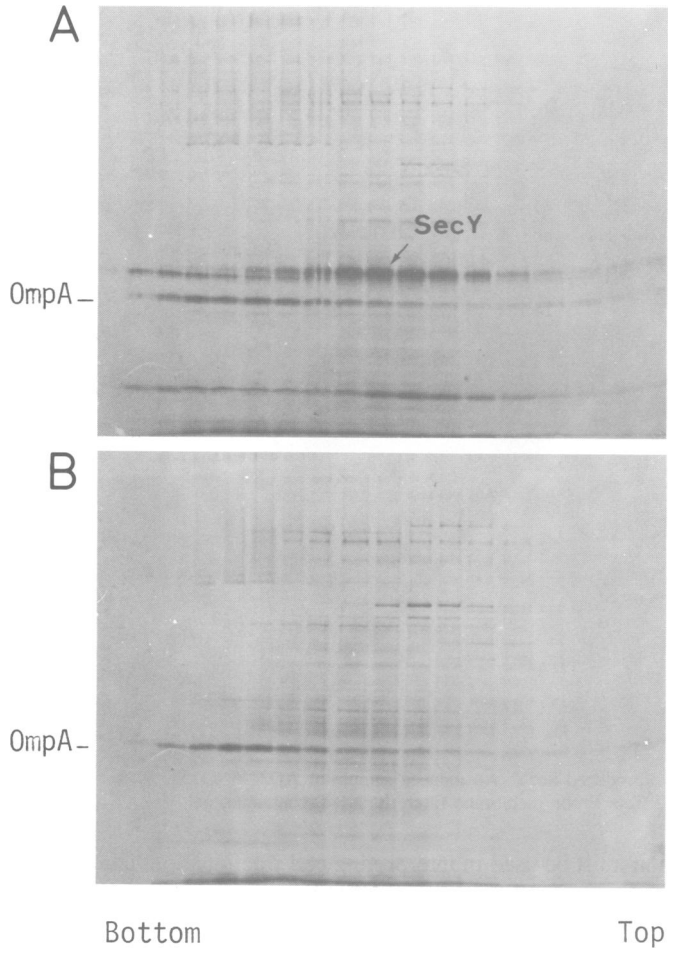

Fig. 3. Localization of the SecY protein as a cytoplasmic (inner) membrane component. Cells of KI269/pNO1573 (**A**) or KI269/pUC8 (**B**) were induced and labeled. Membranes were fractionated by sucrose gradient isopycnic centrifugation in an SW65 rotor at 41 000 r.p.m. for 17 h. Strain KI269 was used in this particular experiment since AD21 did not give a clear separation of the two membranes.

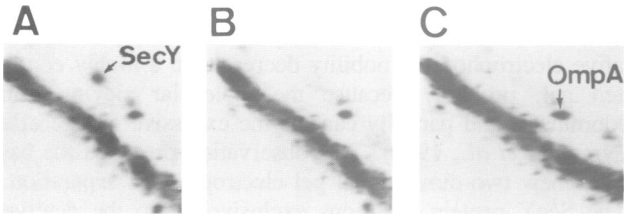

Fig. 4. Behavior of the SecY protein in SDS-SDS two-dimensional gel electrophoresis. Whole-cell extracts were analyzed by SDS-SDS two-dimensional gel. Only the central part of the first-dimensional gel was used. Gels were electroblotted onto a nylon membrane filter (Zeta Probe) before autoradiography. OmpA indicates the putative spot of outer membrane OmpA protein. **A**, induced AD21/pNO1573; **B**, induced AD21/pUC8; **C**, uninduced AD21/pNO1573.

the non-ionic detergent, and, hence, suggesting that it is an integral membrane protein.

A 'three-dimensional' gel system resolves the SecY protein

The SecY protein and certain other integral membrane proteins often show a faster migration on SDS-gel electrophoresis than expected from their molecular weights. The migration becomes slower relative to the reference proteins in a gel with a higher acrylamide concentration (Beyreuther *et al.*, 1980). Thus, the apparent molecular weight of the SecY protein varied from ~32 000 to 42 000, depending upon the gel systems used (Ito, 1984). Similarly, the lactose-carrier protein exhibits apparent mol. wts. of 30 000–46 000 (Beyreuther *et al.*, 1980). We devised a new two-dimensional gel electrophoresis system, taking advantage of this unusual electrophoretic property of the membrane proteins. Samples were first electrophoresed through a low-crosslinked gel, and then subjected to electrophoresis in a second dimension through a more concentrated and more crosslinked gel (see Materials and methods). Because of highly concentrated polyacrylamide used in the second dimension, it was difficult to dry down the gel for autoradiography without cracking. The gels were, consequently, subjected to electro-blotting onto a nylon membrane filter either directly or through another gel containing NP40 (detergent blotting) before autoradiography. In the latter case, we call the whole procedure 'three-dimensional' gel electrophoresis since the detergent blotting step may be regarded as the third dimension.

As shown in Figures 4 and 5, the majority of *E. coli* proteins formed spots along the diagonal line but several proteins were seen off and above the diagonal line. The overproduced SecY protein was clearly identified as one of the spots off the diagonal (Figure 4). The patterns after detergent blotting are shown in Figure 5. The majority of the proteins that aligned on the diagonal passed through the detergent-containing gel and reached the membrane filter (Figure 5A). In contrast, all of the protein spots located off and above the main line, including SecY, partitioned exclusively into the detergent phase (Figure 5B). Thus, this new two (or three)-dimensional gel electrophoresis technique can effectively separate the SecY protein from other proteins.

The chromosome-encoded SecY protein is identifiable as a cytoplasmic membrane protein

We then attempted to identify the SecY protein without overproduction. The ts215 (*rp10215*) mutant with the amber mutation in *rp10* (the gene encoding the L15 protein) was employed. This mutation exerts a strong polar effect on the expression of *secY* (Ito *et al.*, 1984). Cells were pulse-labeled either at 30°C or 42°C. The temperature-sensitive amber suppressor in the mutant strain would be inactivated at 42°C. The whole cell extracts were analyzed by three-dimensional gel electrophoresis. The wild-type strain labeled at either temperature, as well as the mutant strain labeled at 30°C, contained an appreciable amount of a spot at the position where the overproduced SecY protein had been localized (Figure 6A, C and D). This spot was almost absent from the mutant cells labeled at 42°C (Figure 6B). We conclude, therefore, that this spot represents the chromosome-encoded SecY protein.

To study subcellular location of the unamplified SecY protein, and to characterize further the three-dimensional technique, we fractionated the labeled wild-type cells and analyzed each subcellular fraction. Most proteins from the soluble cytoplasmic fraction were present on the diagonal line and were able to pass through the detergent layer (Figure 7A). Periplasmic proteins behaved similarly (data not shown). In contrast, a significant fraction of the proteins from the cytoplasmic membrane formed spots off and above the diagonal line. All of them partitioned exclusively into the detergent phase, producing a characteristic pattern on the detergent-containing gel (Figure 7D). More than 100 spots have been resolved, which presumably represent the integral membrane proteins of *E. coli*. One of these spots has been assigned as SecY (Figure 7D), based on its mobilities as well as on

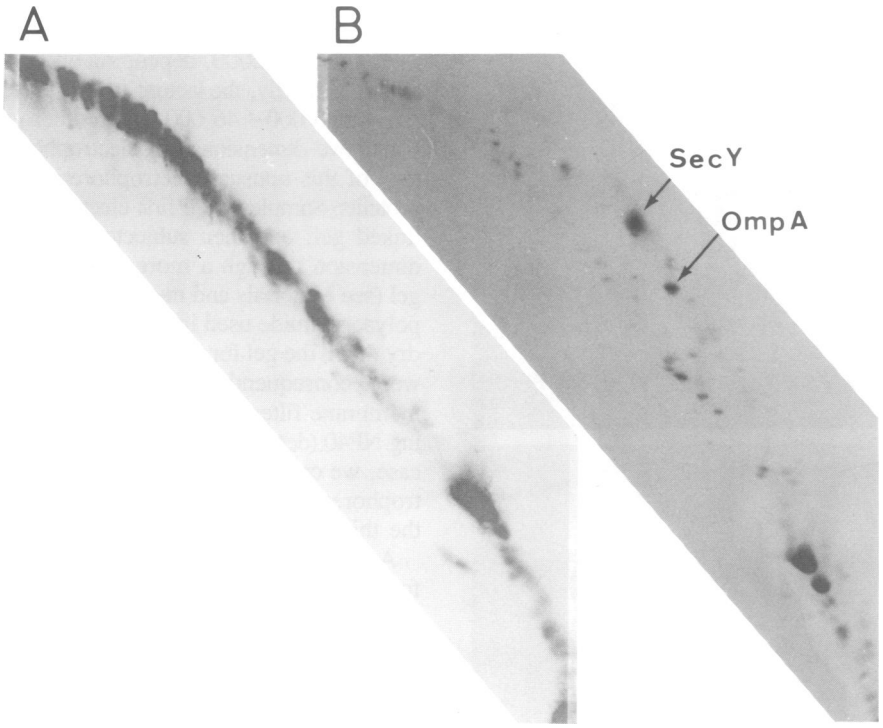

Fig. 5. 'Three-dimensional' gel electrophoresis of a whole-cell extract containing overproduced SecY. An induced sample of AD21/pNO1573 was electrophoresed in SDS-SDS two dimensional gel, followed by detergent blotting. **A**, Zeta Probe membrane filter; **B**, NP40-containing gel.

a comparison with the patterns obtained in Figure 6 (note that the whole cell extracts contained a prominent spot tentatively assigned as the outer membrane OmpA protein; see also Figures 4 and 5). The SecY spot was undetectable from the cytoplasmic (Figure 7A and B) as well as the periplasmic and outer membrane fractions (data not shown). These results establish that the SecY protein, whether overproduced or not, is located in the cytoplasmic membrane as an integral protein.

Discussion

The SecY protein encoded by the *spc* operon *Pst*I fragment is synthesized in large amounts when translation, initiated at the *lacZ* initiation site on the plasmid vector, continues in-frame into the cloned fragment. The results suggest that the expression of *secY* is translationally coupled (Nomura *et al.*, 1984) to the upstream genes, as already suggested previously from the study of the *rpl0215* amber mutation (Ito *et al.*, 1984). To stably maintain such an overproducing plasmid, transcriptional initiation at the *lac* promoter should be kept to a minimum by the absence of endogenous cyclic AMP. In a *cya*$^+$ background, we frequently encountered plasmid derivatives that had lost the capacity to overproduce the SecY protein but not the L15 or the β-galactosidase-S5 fusion proteins. Thus, it appears that even a 'basal' level expression of the SecY protein from such a multi-copy plasmid is harmful to the cell.

Some difference was noted between the proteins overproduced in the u.v.-irradiated maxicells (Ito, 1984) and those overproduced in the growing cell studied here, with respect to their subcellular fractionation behavior. The SecY protein overproduced in the present system was recovered exclusively from the membrane fraction while the L15 protein was present in the soluble cytoplasmic fraction. These proteins, when synthesized in maxicells, fractionated anomalously (Ito, 1984), indicating that caution must be used in interpreting cell fractionation data obtained in the maxicell system; proteins synthesized under such harsh conditions may have non-physiological properties.

The SecY protein has been shown to have some unusual properties characteristic of certain integral membrane proteins (Ito, 1984). First, it is not detectable in SDS gels if a sample has been boiled. Presumably the protein aggregates at the high temperature. Second, its migration in SDS-gel electrophoresis is faster than expected from the molecular weight and differentially affected by the acrylamide concentration. This might be due to an unusually high charge-to-mass ratio of the protein-SDS complex caused by excessive binding of SDS (Beyreuther *et al.*, 1980). The relative electrophoretic mobility decreases in a highly concentrated gel, probably because the molecular sieving effect predominates and partially cancels the excessive charge effect (Beyreuther *et al.*, 1980). This observation provided the basis for our new two-dimensional gel electrophoretic separation.

The SecY protein partitions exclusively into the detergent (NP40) phase during electro-blotting through a detergent layer, suggesting that it is an integral membrane protein. The strong affinity of this protein with the non-ionic detergent (NP40) appears to be the main factor responsible for the failure of this protein to form a well-separated spot in the O'Farrell system (O'Farrell *et al.*, 1977). Although we have not tested the equilibrium system for SecY, our results suggest that there may be many more membrane proteins that have been ignored in the O'Farrell two-dimensional system.

Our alternative two (or 'three') dimensional procedure described here will be useful for hydrophobic membrane proteins. In spite of the simple principle underlying this procedure, it effectively separates membrane proteins. The proteins that form spots off the main diagonal line and that partition into the detergent phase reside mostly in the membrane fraction. Among these

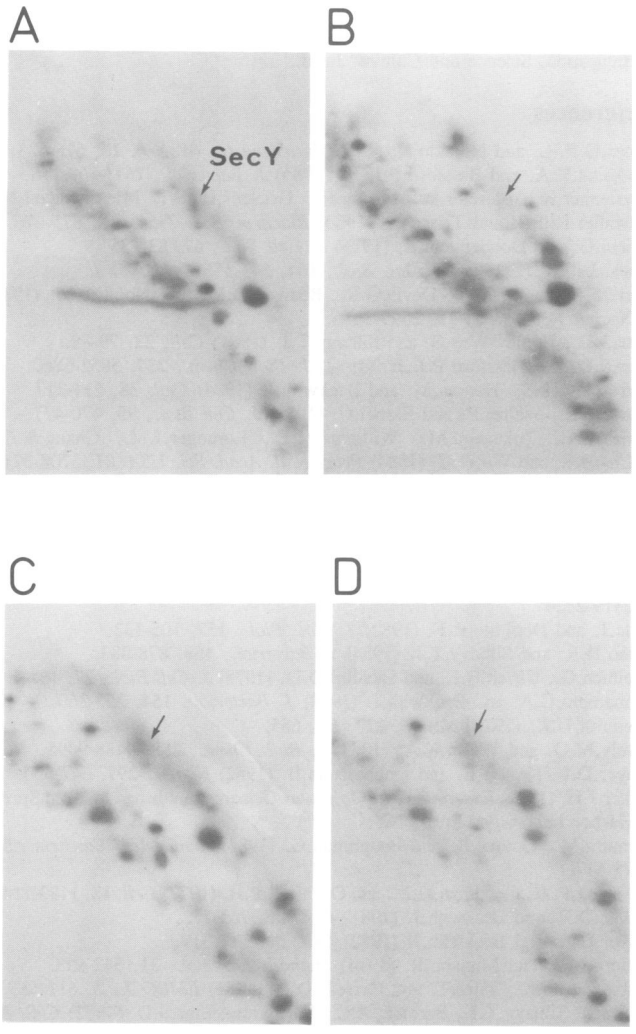

Fig. 6. Identification of the chromosomal-encoded SecY protein. Cells of KI200 (*ts215*; **A** and **B**), as well as KI230 (ts^+; **C** and **D**) were grown first at 30°C (**A** and **C**) and then at 42°C for 1 h (**B** and **D**) before labeling. The whole cell extracts were analyzed by the 'three-dimensional' system: only the patterns of NP40 gels (central part) are presented.

membrane proteins, we have identified the SecY protein in its physiological cellular amount. We have also identified the lactose carrier protein in this system (data not shown). This new electrophoretic procedure should be applicable to membrane proteins from a variety of organisms.

These studies indicate that the SecY protein is an integral membrane protein located in the cytoplasmic membrane of *E. coli*. The hydropathy analysis (Kyte and Doolittle, 1982) showed that there are ~10 hydrophobic segments in this protein (Cerretti *et al.*, 1983), suggesting that it spans the membrane several times. Throughout the eukaryotes and prokaryotes, only a few integral membrane proteins are known to be involved in protein translocation. These include the signal (leader) peptidases from *E. coli* (Wolfe *et al.*, 1983; Innis *et al.*, 1984; Yu *et al.*, 1984) and from an animal cell (Lively and Walsh, 1983), as well as the SRP (signal recognition particle)-receptor protein from dog pancreas (Meyer *et al.*, 1982; Gilmore *et al.*, 1982). The SRP-receptor has a large (60 000 dalton) hydrophilic domain exposed to the cytoplasm. Thus it seems unlikely that the SecY protein, which lacks a hydrophilic segment of a comparable size, is a bacterial counterpart of the SRP receptor protein. The ribophorins are integral membrane proteins specifically found in the rough endoplasmic reticulum (Kreibich *et al.*, 1978), and their role as ribosome-binding proteins in the vectorial translocation system was implicated. There is a possibility that the SecY protein is analogous to ribophorins. In view of the highly amphiphilic nature of the SecY protein, and the likelihood that it traverses the membrane several times, it is also conceivable that the protein forms a proteinaceous pore in the membrane for the passage of polypeptides. Such pores were originally postulated in the signal hypothesis (Blobel and Dobberstein, 1975).

The work by Silhavy and others has shown that the *prlA* mutations, which suppress the changes in the signal sequence in an allele-specific manner (Emr *et al.*, 1981; Emr and Bassford, 1982), are located in the DNA fragment included within the *secY* gene (Shultz *et al.*, 1982). Our results on the SecY protein and those of Silhavy *et al.* on the *prlA* mutations suggest that signal recognition in bacterial protein export may be carried out by an integral membrane protein, the SecY protein. In view of the apparent evolutional conservation of the protein translocation reac-

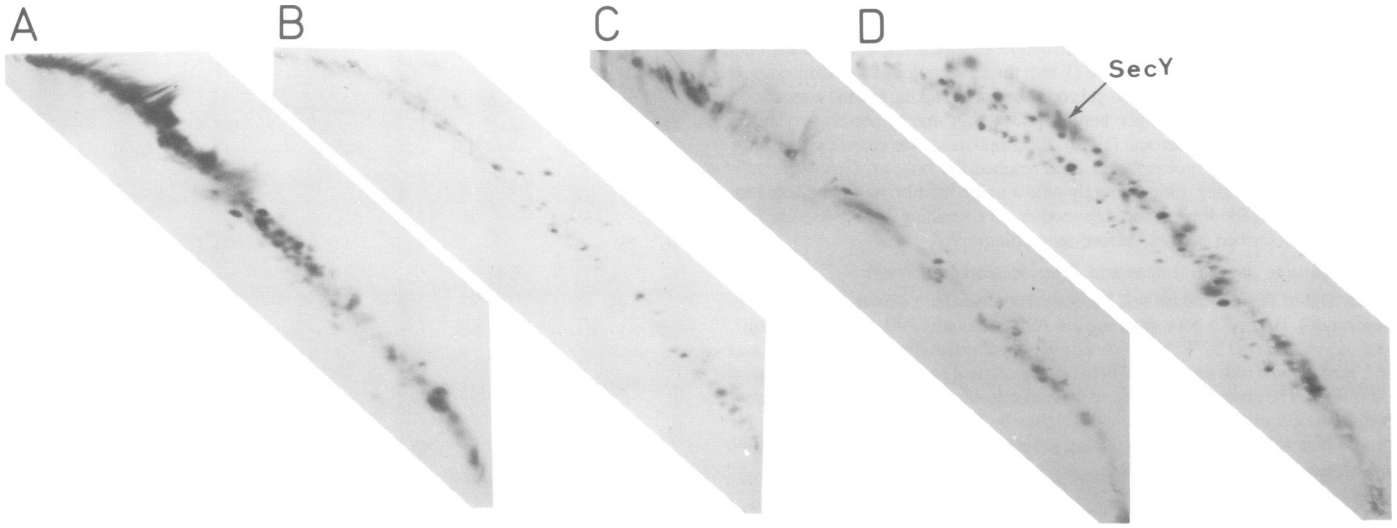

Fig. 7. Three-dimensional gel electrophoresis of the cytoplasmic and cytoplasmic membrane proteins. Strain MC4100 was grown at 30°C with maltose (0.4%), labeled, and fractionated into the four subcellular fractions. Samples were analyzed by SDS-SDS two-dimensional gel electrophoresis and detergent blotting. **A** and **B**, Zeta Probe filter and NP40-containing gel, respectively, for the cytoplasmic fraction. **C** and **D**, Zeta Probe filter and NP40-containing gel for the cytoplasmic (inner) membrane fraction.

tion, there may be multiple steps in signal recognition, one involving an SRP-like factor in the cytosol (Walter *et al.*, 1984), and another involving the transmembrane SecY protein. Finally, we point out that a protein analogous to SecY may be generally present in translocation-competent membranes of eukaryotes and prokaryotes.

Materials and methods

Bacterial strains and plasmids

The *E. coli* K12 strains MC4100 (Δ*lac araD thiA rpsL relA*) and CSH26 (Δ*pro-lac thi*) were described by Casadaban (1976) and Miller (1972), respectively. KI200 [MC4100, *rpl0215*(amber) Tn10 (φ80*sus2*p*supF*^{ts6})] and KI230 [thiA (φ80*sus2*p*supF*^{ts6})] were described previously (Ito *et al.*, 1984). For constructing AD21 (CSH26, *cya283*), F′ *lacI*^{q} *Z*^{M15} *Y*$^{+}$ *pro*$^{+}$ (provided by M.Nomura) was transferred to CHS26, *ilv*::Tn10 was introduced by P1 transduction (using a *ilv*::Tn10 strain provided by S.Brown as a donor), and one of the resulting transductants was converted to *cya283* by selecting *ilv*$^{+}$ transductants (using a *cya283* strain provided by M.Imai as a donor). Strain KI269 (MC4100, *cya283*/F′*lacI*^{q} *Z*$^{+}$ *Y*$^{+}$ *pro*$^{+}$) was constructed in the same way except that F′*lacI*^{q} *Z*$^{+}$ *Y*$^{+}$ *pro*$^{+}$ (provided by M.Nomura) was introduced at the last step.

Plasmid PnO1573 was constructed by M.Wittekind and M.Nomura. It is a derivative of pUC8 (Vieira and Messing, 1982), with the 2.8-kb *Pst*I-*Pst*I fragment of the *spc* operon inserted at the *Pst*I site in the 'correct' orientation. pKY16 is a derivative of pUC9, with the *Pst*I-*Pst*I insert of pNO1573 recloned into the *Pst*I site (strain AD21 was used as a host).

Media, labeling and sample preparation

Cells were grown in minimal medium E (Vogel and Bonner, 1956) supplemented with thiamine (2 μg/ml), 18 amino acids (20 μg/ml each) other than methionine and cysteine, and a carbon source (0.4% glucose, unless otherwise indicated). The temperature was 37°C unless otherwise stated. Ampicillin (50 μg/ml) was included for plasmid-bearing strains when appropriate. Induction of the lactose promoter was initiated 6 min before labeling by adding 1 mM IPTG and 5 mM cyclic AMP. Cells (reading 40–80 in a Klett colorimeter with number 54 filter) were labeled with 5–34 μCi/ml of [^{35}S]methionine (1100 Ci/mmol, New England Nuclear) for 3 min. Whole cell extracts for SDS-gel electrophoresis were prepared by the lysozyme-freeze-thaw method described previously (Ito, 1984). Cell fractionation was carried out as described (Ito *et al.*, 1977) except that sucrose gradient separation of inner and outer membranes was by the method of Osborn and Munson (1974), and that 1 mM phenylmethyl sulfonylfluoride and 3 mM tosyl-L-lysine chloromethylketone were added to the harvested culture and to the buffer used for cell disruption. Samples were mixed with an equal volume of 2-fold concentrated sample buffer, followed by incubation at 37°C for 3 min, before electrophoresis (Ito, 1984).

SDS-polyacrylamide gel electrophoresis

The modified Laemmli (1970) system with 15% polyacrylamide and 0.12% N,N′-methylene-bis-acrylamide (Ito, 1984) was used for one dimensional electrophoresis.

SDS-SDS two-dimensional gel electrophoresis

For SDS-SDS two-dimensional gel electrophoresis (see text), a lane of the one-dimensional SDS gel described above (1 mm thick; 8 cm long) was excised, and placed on top of a second dimension gel composed of a stacking gel and a separation gel (14 x 15 x 0.1 cm) of 20% acrylamide-0.53% N,N′-methylene-bis-acrylamide and the buffer system of Laemmli (1970). Electrophoresis was carried out (30 mA constant current) until bromophenol blue reached the bottom. Gels were subjected to electro-blotting, either directly onto a nylon membrane filter (Zeta Probe, obtained from Bio Rad) or through another gel containing 2% NP40 (detergent blotting, as described below), before autoradiography.

Protein blotting through a detergent layer (detergent blotting)

The details of this procedure will be described elsewhere (Ito and Akiyama, in preparation). Briefly, a gel containing 2% NP40 (Nonidet P40, product of Shell), 10% acrylamide, 0.27% N,N′-methylene-bis-acrylamide, 2.5 mM Tris and 19.2 mM glycine was sandwiched between the original gel and a Zeta Probe membrane filter. The sandwich was placed in an electro-blotting apparatus, and electro-blotted in 2.5 mM Tris, 19.2 mM glycine buffer (pH 8.4) at 20 V/cm for 20–24 h at 4°C. The NP40-containing gel and the membrane filter were dried and exposed to Fuji X-ray films.

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References

Ames,G.F.-L. and Nikaido,K. (1976) *Biochemistry (Wash.)*, **15**, 616-623.
Bankaitis,V.A. and Bassford,P.J.,Jr. (1985) *J. Bacteriol.*, **161**, 169-178.
Beyreuther,K., Bieseler,B., Ehring,R., Griesser,H.-W., Mieschendahl,M., Müller-Hill,B. and Triesch,I. (1980) *Biochem. Soc. Trans.*, **8**, 675-676.
Blobel,G. and Dobberstein,B. (1975) *J. Cell Biol.*, **67**, 835-851.
Casadaban,M.J. (1976) *J. Mol. Biol.*, **104**, 541-555.
Cerretti,D.P., Dean,D., Davis,G.R., Bedwell,D.M. and Nomura,M. (1983) *Nucleic Acids Res.*, **11**, 2599-2616.
Emr,S.D., Hanley-Way,S. and Silhavy,T.J. (1981) *Cell*, **23**, 79-88.
Emr,S.D. and Bassford,P.J.,Jr. (1982) *J. Biol. Chem.*, **257**, 5852-5860,
Ferro-Novick,S., Honma,M. and Beckwith,J. (1984) *Cell*, **38**, 211-217.
Gilmore,R., Walter,P. and Blobel,G. (1982) *J. Cell Biol.*, **95**, 470-477.
Innis,M.A., Tokunaga,M., Williams,M.E., Loranger,J.M., Chang,S.-Y., Chang,S. and Wu,H.C. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3708-3712.
Ito,K., Sato,T. and Yura,T. (1977) *Cell*, **11**, 551-559.
Ito,K., Wittekind,M., Nomura,M., Shiba,K., Yura,T., Miura,A. and Nashimoto,H. (1983) *Cell*, **32**, 789-797.
Ito,K. (1984) *Mol. Gen. Genet.*, **197**, 204-208.
Ito,K., Cerretti,D.P., Nashimoto,H. and Nomura,M. (1984) *EMBO J.*, **3**, 2319-2324.
Kyte,J. and Doolittle,R.F. (1982) *J. Mol. Biol.*, **157**, 105-132.
Kiino,D.R. and Silhavy,T.J. (1984) *J. Bacteriol.*, **158**, 878-883.
Kreibich,G., Ulrich,B.L. and Sabatini,D.D. (1978) *J. Cell Biol.*, **77**, 464-487.
Kumamoto,C.A. and Beckwith,J. (1983) *J. Bacteriol.*, **154**, 253-260.
Laemmli,U.K. (1970) *Nature*, **227**, 680-685.
Lively,M.O. and Walsh,K.A. (1983) *J. Biol. Chem.*, **258**, 9488-9495.
Meyer,D.I., Krause,E. and Dobberstein,B. (1982) *Nature*, **297**, 647-650.
Miller,J.H. (1972) *Experiments in Molecular Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Nomura,M., Gourse,R. and Baughman,G. (1984) *Annu. Rev. Biochem.*, **53**, 75-117.
O'Farrell,P.Z., Goodman,H.M. and O'Farrell,P.H. (1977) *Cell*, **12**, 1133-1142.
Oliver,D.B. and Beckwith,J. (1981) *Cell*, **25**, 765-772.
Oliver,D.B. and Beckwith,J. (1982) *Cell*, **30**, 311-319.
Osborn,M.J. and Munson,R. (1974) *Methods Enzymol.*, **31**, 642-653.
Shiba,K., Ito,K., Yura,T. and Cerretti,D.P. (1984) *EMBO J.*, **3**, 631-635.
Shultz,J., Silhavy,T.J., Berman,M.L., Fiil,N. and Emr,S.D. (1982) *Cell*, **31**, 227-235.
Vieira,J. and Messing,J. (1982) *Gene*, **19**, 259-268.
Vogel,H.J. and Bonner,D.M. (1956) *J. Biol. Chem.*, **218**, 97-106.
Walter,P., Gilmore,R. and Blobel,G. (1984) *Cell*, **38**, 5-8.
Wolfe,P., Wickner,W. and Goodman,J. (1983) *J. Biol. Chem.*, **258**, 12073-12080.
Yu,F., Yamada,H., Daishima,K. and Mizushima,S. (1984) *FEBS Lett.*, **173**, 264-268.

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