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MINI-REVIEW

Structure, Function, and Biogenesis of SecY, an Integral Membrane Protein Involved in Protein Export

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Abstract

The *E. coli secY* (*prlA*) gene, located in the operator-distal part of the *spc* ribosomal protein operon, codes for an integral membrane protein, SecY. The phenotypes of temperature-sensitive and cold-sensitive mutations in *secY* suggest that the SecY protein plays an essential role *in vivo* to facilitate protein translocation, whereas the *prlA* mutations in this gene suggest that SecY may interact with the signal sequence of translocating polypeptides. SecY contains most probably six cytoplasmic and five periplasmic domains, as well as 10 transmembrane segments. Such membrane-embedded structure may confer the SecY protein a "translocator" function, in which it provides a proteinaceous pathway for passage of secreted as well as membrane proteins. Results obtained by *in vitro* analyses of the translocation reactions, as well as some new phenotypes of the *secY* mutants, are consistent with this notion. Possible interaction of SecY with other secretion and chaperone-like factors is also discussed.

Key Words: *sec* mutant; *secY*; *prlA*; protein export; membrane protein; export signal; suppression; heat-shock protein.

Introduction

Secreted and membrane proteins follow complex folding pathways in their post-synthetic process of translocation across or integration asymmetrically into the hydrophobic milieu of the membrane. The signal (or leader) sequence carried on a presecretory protein determines whether the polypeptide portion

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that follows it is to be translocated across the membrane. Integration of a membrane protein is also triggered by a signal peptide equivalent; the uncleavable (or internal) version of signal peptide generates a transmembrane segment of N-in C-out orientation, whereas a "stop-transfer sequence" following a signal, as well as a "start-stop" sequence, are responsible for membrane anchorage in the other (N-out C-in) orientation (for review, see von Heijne, 1988).

The translocation process is assisted by some proteinaceous cellular factors, some of which reside in the cytoplasm while others are associated with the membrane. The signal peptide is thought to be recognized by cytoplasmic factors, such as the eukaryotic signal recognition particle which targets the translation complex to the membrane (for review, see Walter and Lingappa, 1986). Other cytoplasmic factors such as yeast Hsp70s (for review, see Dashaies *et al.*, 1988), *E. coli* SecB (Collier *et al.*, 1988), GroE (Bochkavera *et al.*, 1988), and Trigger factor (Crooke *et al.*, 1988) maintain the precursor polypeptide in a conformation devoid of tight folding and suitable for the post-translational transit through the membrane (Randall and Hardy, 1989).

The *E. coli* SecA protein (Oliver and Beckwith, 1982) is a peripheral membrane factor with an ATPase activity (Lill *et al.*, 1989), which should be related to the utilization of ATP, an energy source for translocation.

In contrast to the early processes that are aided by factors in the cytoplasm or on the membrane surface, very little is known about the trans-bilayer movement of polypeptides. A central question here is whether the translocating polypeptide moves through a pathway provided by some proteinaceous catalyst (translocator) within the membrane. It has repeatedly been proposed that protein translocation should occur via a tunnel formed by some proteins within the membrane (Blobel and Dobberstein, 1975; Singer *et al.*, 1987), but experimental evidence for this notion is still lacking and the possibility remains that proteins extrude directly through the lipid phase of the membrane. Thus, it is important to identify integral membrane proteins that facilitate translocation of other proteins. In *E. coli*, the gene products of *secY* (see below) and, more recently, of *secE* (Schatz *et al.*, 1989) have been shown to be integral membrane factors for translocation.

After the trans-bilayer movement, a secretory protein must be released from the membrane and folded into water-soluble conformation, whereas a membrane protein should stably be anchored into the lipid phase of the membrane where further intra- or intermolecular associations may follow. These latter processes are left almost entirely for future investigations.

This review summarizes the work conducted in our laboratory as well as in other laboratories, concerning the structure and function of the *secY* gene product.

The Gene *secY* Is in the *spc* Ribosomal Protein Operon

Emr *et al.* (1981) first reported mutations affecting an aspect of protein export and mapping near the ribosomal protein gene cluster at 72 min on the *E. coli* chromosome. These mutations, termed *prlA*, suppressed signal sequence mutations in *lamB* (for an outer membrane protein). Schultz *et al.* (1982) subsequently showed that *prlA* was probably located in a previously unidentified locus at the operator-distal part of the *spc* ribosomal protein operon.

Our studies on *secY* stemmed from our interest in the relationships between protein synthesis and protein export. In order to define the possible roles of ribosomes in export, we screened temperature-sensitive (Ts) mutants obtained by localized mutagenesis of the ribosomal protein loci, including *spc*. Pleiotropic protein export mutants were thus isolated and shown to have genetic lesion linked to *spc*. Complementation tests using various plasmids indicated that the gene responsible for the export defect was the open reading frame "Y" within the *spc* operon which was sequenced by Cerretti *et al.* (1983). This result led to our proposal that this gene be termed *secY* (Ito *et al.*, 1983; Shiba *et al.*, 1984a). One of such mutants (*ts215*) proved subsequently to be an amber mutant in the preceding L15 (*rplO*) gene; when the Ts amber suppressor present in this mutant was inactivated, the mutation exerted a polar effect on the expression of *secY* (Ito *et al.*, 1984). Like the ribosomal protein cistrons in this operon (Nomura *et al.*, 1984), *secY* should be translationlly coupled to the upstream cistrons. At the operator-distal side of *secY*, there is another open reading frame (called X) which terminates the operon (Cerretti *et al.*, 1983). X is now known as *rpmJ* and to code for a new ribosomal large sutunit protein (L36) (Wada and Sako, 1987; Ueguchi *et al.*, 1989). When the *rpmJ* region of the chromosome is disrupted by some untranslated sequence, the expression of *secY* becomes impaired. This "retro" effect may be due to the instability of the *secY* part of the messenger RNA. The *rpmJ* gene itself does not seem to be important for protein export or cell growth (Ueguchi *et al.*, 1989).

It is intriguing to ask why *secY* for an integral membrane protein belongs to this otherwise ribosomal protein operon. Possibly, the coordinate expression of *secY* and ribosomes is of some evolutional advantage, although the level of *secY* expression appears to be about 1/5 the ribosomal protein levels (Ueguchi *et al.*, 1989). It should be noted that *secE* for another integral membrane translocation factor is also within a cluster of genes at 90 min for translation factors (Schatz *et al.*, 1989).

Temperature-Sensitive and Cold-Sensitive Mutants of *secY*

As mentioned above, the *secY24*(Ts) mutant was isolated from Ts mutants obtained by localized mutagenesis, without involving a selection

step (Shiba *et al.*, 1984a). In contrast, no *secY* mutant was selected by the procedure of Oliver and Beckwith (1981), which allowed them to isolate the *secA* and the *secB* mutants. Such a selection system may have potential limitations in obtaining mutants of growth-essential genes, since it relies on some partially defective phenotypes under the "permissive" conditions, and the spectrum of mutants to be selected can be biased by the selective pressure. To assess the importance of *secY*, we tried a more simple (but tedious) way of screening a random collection of Ts mutants for precursor accumulation using immunoblotting. This screening indeed yielded one additional *secY* Ts mutant (*secY100*) out of some 600 Ts mutants (Ito *et al.*, 1989). Recently, Riggs *et al.*, (1988) were able to isolate some cold-sensitive (Cs) *secY* mutants by another selection based on the enhanced exporession of *secA* under the conditions of lowered protein export.

We have sequenced the Ts and Cs *secY* mutations (see Fig. 1). Mutation *secY24*(Ts) causes Gly to Asp change in the cytoplasmic loop 4 (Shiba *et al.*, 1984a; see below for the topological model of SecY in the membrane), whereas *secY39*(Cs) and *secY40*(Cs) are both in the cytoplasmic loop 5 with Arg to His and Ala to Ser changes, respectively. *SecY100* contains three amino acid changes, two of them in transmembrane segments and one of them in a periplasmic loop (Ito *et al.*, 1989); it is not known whether all of them are responsible for the phenotype of this mutant.

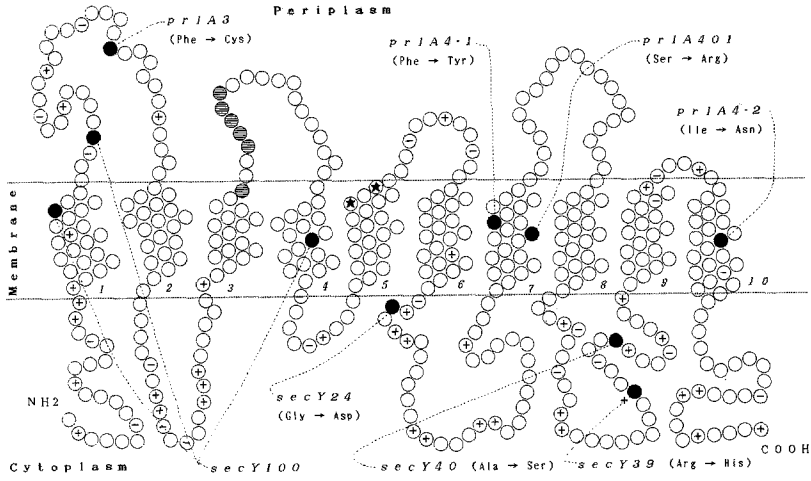

Fig. 1. A model for the topological disposition of the SecY protein in the membrane. Circles represent amino acid residues starting from the residue 2 of the primary translation product. Transmembrane segments are numbered. The filled circles represent the sites of mutational alterations as indicated (see text for the references). The hatched circles correspond to the sequence also found in the yeast gene SRH1.

The Ts mutants accumulate precursor forms of various exported proteins within the cell, that is, in the location which is inaccessible by externally added proteases. Thus, the defect is in the translocation step rather than in the signal cleavage reaction (Shiba *et al.*, 1984a). The precursor molecules accumulated can undergo slow post-translational export, and more rapid export when supplied with the wild-type SecY protein from a plasmid (Bacallao *et al.*, 1986). Thus, SecY can stimulate post-translational translocation, and the procursor proteins aborted in the *secY* mutant do not irreversibly lose their competence for translocation.

The *sec*Ts mutants, including *secY24* and *secA51*, often requires 1–2 hr of incubation at the nonpermissive high temperature before their phenotypes of defective protein export are fully expressed. One interpretation of these results is that these mutations temperature-dependently impair the process of formation of active export apparatus rather than the catalytic reactions of the gene products. This poses some problem in designing experiments *in vivo* and *in vitro*, as well as in their interpretations. We found a different situation in one Cs mutant, *secY39*. In this mutant, temperature shift-down causes immediate retardation in protein export (T. Baba and K Ito, unpublished results). Such rapid response may suggest that the site altered by *secY39* is more directly involved in the catalytic mechanism of the protein. This mutant thus provides better systems for analysis of reaction parameters of protein translocation both *in vivo* and *in vitro*.

SecY May Interact with Signal Sequence

As already mentioned, the *prlA* mutations restore export of secretory proteins whose signal sequence is otherwise defective because of a mutation in the hydrophobic core region. These mutations are dominant over the wild-type allele, and their mutated bases have been assigned within the *secY* sequence (see Fig. 1), mostly within the transmembrane segments (Stader *et al.*, 1986; Sako and Iino, 1988). The extragenic suppression of the signal sequence mutation is not strictly allele-specific, but does show some mutation-specific variation (Emr and Bassford, 1982). In this context, it should be remembered that the signal sequence themselves are not conserved, and their recognition may not necessarily be sequence-specific to the extent expected from the classical notion of "allele specificity".

The *prlA* mutations usually do not affect export of wild-type envelope proteins. However, one interesting new phenotype of *prlA* has recently been reported; *prlA* causes defective translocation of some proteins, such as staphylokinase, derived from Gram-positive bacteria (Iino and Sako, 1988; Muller *et al.*, 1989). Sako and Iino (1988) have further shown that the *prlA4*

mutant contains two mutations, one (*prlA4-1*) in the transmembrane segment 7 and the other in the last transmembrane segment. The former mutation is responsible for the rejection of staphylokinase, while the latter is responsible for the suppression of signal sequence mutations of *E. coli* proteins. The *prlA401* mutation (Bankaitis and Bassford, 1985) is in the transmembrane 7 and is responsible for both phenotypes. The *prlA3* mutation is in a periplasmic loop and is responsible for the suppression of signal mutations. Mutations of staphylokinase overcoming the *prlA* block can, in turn, occur in the signal peptide part of the enzyme. Based on the last observation, Iino and Sako (1988) proposed that SecY may recognize a bent structure of signal peptide. These studies of the *prlA* mutations suggest that the *prlA* (*secY*) gene product interacts directly with the signal peptide of the translocating molecule, although biochemical evidence is not yet available.

Recently, *secY* homologs have been searched in eukaryotes, and a yeast chromosomal DNA fragment was found to hybridize with *secY*, and a gene containing this fragment has been cloned and sequenced (Y. Amaya, A. Nakano, K. Ito, and M. Mori, manuscript submitted). The sequence has no overall similarity to *secY*, except for a 27-bp region, of which 21 bases are matched perfectly. Intriguingly, this *S. cerevisiae* gene, termed SRH1, proved most likely to be the yeast homolog of the 54-kDa signal sequence-binding subunit of the signal-recognition particle (Romisch *et al.*, 1989; Bernstein *et al.*, 1989). The limited SecY region that is homologous to SRH1 encodes $\underline{G}^{138}\underline{LPNMPGM}$ (homologous residues underlined), which is located in SRH1 within the unusually methionine-rich region. The possible role of methionine-rich sequences in signal sequence recognition has been proposed by Berstein *et al.*, (1989). Amazingly, the above sequence, as well as the *prlA3* mutation, face the periplasmic side in our topology model of SecY (Fig. 1). The existence of the short SecY segment homologous to SRH1, along with the *prlA* mutations, could provide clues to our understanding of the possible signal-recognizing ability of the SecY protein.

Identification and Characterization of the SecY Protein

The product of *secY* was expected to be a hydrophobic and basic protein of about 49,000 kDa, but its identification required some caution. We first identified it in cells carrying a SecY-overproducing plasmid, and showed that this protein shares some peculiar properties with hydrophobic membrane proteins such as the lactose permease (LacY) (Ito, 1984; Akiyama and Ito, 1985). It is not electrophoretically identifiable after boiling in SDS, presumably due to aggregation. It migrates in SDS PAGE faster than expected from the molecular mass. Its migration in SDS-PAGE is differentially affected by

acrylamide concentrations, enabling its separation as a spot off the diagonal line by two-dimensional SDS-PAGE using two different acrylamide concentrations ("SDS–SDS two-dimensional PAGE"). SecY partitions to the nonionic detergen (NP40)-containing gel in our new blotting procedure called "detergent blotting" (Ito and Akiyama, 1985), consistent with its being a hydrophobic and integral membrane protein. Presumably, the strong interaction between SecY and the nonionic detergent prevents its movement through the detergent-containing polyacrylamide gel, and also its separation in the O'Farrell type two-dimensional gel electrophoresis (Akiyama and Ito, 1985). We isolated chemical amounts of SecY from the spot of SDS–SDS two-dimensional gel and determined its amino-terminal sequence, confirming its identity; SecY does not undergo amino-terminal processing except for the removal of the initiator methionine (Akiyama and Ito, 1986). The new electrophoretic methods mentioned above also enabled us to identify the SecY protein in the wild-type cytoplasmic membrane without amplification (Akiyama and Ito, 1985).

Localization and Topology of the SecY Protein

Both the amplified and unamplified SecY fractionate with the cytoplasmic (inner) membrane (Akiyama and Ito, 1985). SecY in the wild-type cell is not extractable from the membrane by alkali, indicating that it is integrally associated with the lipid bilayer (Y. Akiyama and K. Ito, unpublished results). The hydropathic analysis of the SecY amino acid sequence revealed 10 hydrophobic segments whose hydrophobicity and length qualify them as membrane-spanning segments (Cerretti *et al.*, 1983; Akiyama and Ito, 1987). Disposition of SecY in the membrane has been studied by protease digestion patterns, as well as by analysis of a series of SecY-PhoA fusion proteins (Akiyama and Ito, 1987) constructed by transposition of Tn*phoA*. In the latter approach developed by Manoil and Beckwith (1986), the mature sequence of alkaline phosphatase (PhoA) was attached to various regions of SecY. Fusions at periplasmic domains were expected to produce SecY-PhoA fusion proteins with their PhoA part facing the periplasm, whereas fusions at cytoplasmic regions should produce internalized PhoA moiety. These analyses identified five periplasmic regions and at least two cytoplasmic domains of SecY. Taken together, the model shown in Fig. 1 is the most probable representation of the topological disposition of SecY. Thus, SecY consists of six cytoplasmic, five periplasmic, and 10 transmembrane regions (Fig 1).

Our analysis using the PhoA fusions showed that the alternate SecY transmembrane segments (starting from the most N-terminal one) act as an export signal for translocation of PhoA attached carboxy-terminally. The

PhoA fusion method relied on the notion that the PhoA mature sequence faithfully reflects the preceding export signal and PhoA can only be enzymatically active after export to the perisplasm (Manoil and Beckwith, 1986). We used not only the enzymatic activity but also more direct assays to determine localization of the PhoA moiety (Akiyama and Ito, 1989). Protease digestion of inact spheroplasts cleaved the fusion protein around the junction point when it is periplasmically exposed, while cytoplasmic fusions are inaccessible to the protease. The correctly folded PhoA itself is resistant to proteases. Thus, trypsin treatment of detergent-solubilized extract yielded indigestible PhoA fragment when it was periplasmic, while the internalized (and hence unfolded) PhoA was completely digested. These assays enables us not only to confirm the disposition of the PhoA part (and hence the SecY part just preceding it) of the fusion proteins, but also to follow, by pulse-chase experiments, the *in vivo* processes of PhoA export that was aided by the export signals carried within SecY. This mode of PhoA export is similar to its export aided by a normal signal sequence, in that it is rapid and dependent on the function of the normal $SecY^+$ function provided *in trans* (Akiyama and Ito, 1989).

In the course of characterization of the SecY-PhoA fusions, we were able to identify a cryptic leader peptidase cleavage site within SecY just following the fifth transmembrane segment (Fig. 1), which becomes efficiently cleaved when PhoA follows it (Y. Akiyama and K. Ito, manuscript submitted). *In vivo* experiments using the *lep*(Ts) mutant (Inada *et al.*, 1989), *in vitro* experiments using the purified leader peptidase, and site-directed mutagenesis experiments established that the Ala^{202}-Ile-Ala sequence located in the immediate C-terminal vicinity of the transmembrane segment 5 (Fig. 1) was recognized by the leader peptidase. Thus, this internal transmembrane sequence potentially fulfills the two important aspects of the signal peptide functions, translocation and cleavage. The existence of potential signal peptidase cleavage site in integral membrane proteins has been reported also in some eukaryotic systems (Lipp and Dobberstein, 1986; Schmid and Spiess, 1988). However, there has been no evidence that the potential cleavage sites are used in the wild-type membrane proteins.

Overproduction of SecY

For biochemical studies of SecY, it is important to overproduce and purify SecY which can then be reconstituted into artificial membranes. To achieve a high-level oversynthesis of SecY, cloning should be done such that the accompanying upstream *rplO* fragment is translated (Akiyama and Ito, 1985) or the *secY* is directly fused to the initiation codon of an efficiently

translated gene such as *lacZ* (Y. Akiyama and K. Ito, unpublished results). SecY-overexpressing plasmids thus constructed were very unstable and tight repression of the gene was essential for their maintenance. In other words, overproduction of SecY is highly toxic to the cell. Indeed, SecY synthesis above certain levels caused immediate stop in cell growth. Interestingly, overproduction of some truncated forms of SecY dissipated the proton-motive force. In addition, oversynthesized SecY is very unstable *in vivo* (Akiyama and Ito, 1986). We observed that the growth of the cell and the stability of the oversynthesized SecY were almost mutually exclusive. We should overcome these difficulties before obtaining a high-level producer of SecY.

In Vitro Analysis of the SecY Function

The importance of the *secY* function has been demonstrated *in vitro*. Inverted plasma membrane vesicles prepared from the *secY24*(Ts) mutant grown at high temperature did not support translocation of a secretory precursor (Bacallao *et al.*, 1986). Fandl and Tai (1987) showed that the mutant membrane was heat-inactivated *in vitro*. Watanabe and Blobel (1989) showed that the Fab fragments of anti-SecY synthetic peptide inhibited protein translocation *in vitro*. Although Watanabe and Blobel (1989) concluded that the antibody-mediated inhibition was at the binding of the precursor to the membrane, this requires further evidence because their experiments did not define conditions for precursor binding without translocation. In any case, these *in vitro* results are consistent with SecY being a multi-path membrane protein, and taken together with the *in vivo* data establish that the SecY function is essential for efficient translocation of *E. coli* envelope proteins across the cytoplasmic membrane.

The *in vitro* study of Tai and coworkers also showed that the inactivated membrane vesicles from the *secY* mutant could be reativated by an excess of the SecA protein (Fandl *et al.*, 1988). Lill *et al.*, (1989) demonstrated that the SecA ATPase is markedly stimulated by membrane vesicles containing functional SecY and a translocation-competent precursor protein. The ATPase activity was also inhibited by anti-SecY serum. Thus, SecY and SecA may interact with each other. However, genetic evidence for such interaction is still lacking, since the *prlA1012* mutation, originally described as suppressing a *secA*(Ts) mutation (Brickman *et al.*, 1984), does not lie within the *secY* gene (T. Taura, E. Brickman, J. Beckwith, and K. Ito, unpublished results).

Concerning the *in vitro* translocation experiments, we encountered an observation which calls for some caution; based on our immunoblotting

results, a significant proportion of SecY in typical preparations of membrane vesicles is cleaved into smaller fragments by the OmpT protease present in the membrane fraction (Y. Akiyama and K. Ito, unpublished results). Eliminating the protease activity genetically (Y. Akiyama and K. Ito, unpublished results) will be important in manipulating membrane vesicles or purifying SecY. We are currently setting up *in vitro* translocation systems with vesicles containing only the intact SecY protein.

Some New Phenotypes of the *secY* Mutants

The *secY24*(Ts) mutant exhibits some notable phenotypes at the "permissive" low temperature, which may provide clues for the possible function of SecY as translocator. Protein export in the *secY24* mutant at the "permissive" low temperature is hypersensitive to overproduction of an exportable protein such as β-lactamase, as well as to a low-level synthesis of the MalE-LaZ hybrid protein (Ito *et al.*, 1989). The *secY24* mutant is more subject to such inhibition than other *sec* mutants. Assuming that SecY is an important component of translocator, these results imply that the *secY24* mutation alters the SecY translocator such that it is extremely sensitive to jamming by the hybrid protein or by the increased influx of polypeptide molecules to be exported. Beiker and Silhavy (1989) also suggested that SecY (PrlA) may be the site of action of the export-jamming LacZ hybrid proteins. They showed that overproduction of PrlA overcame the inhibition, and, using combinations of *prlA4* and the hybrid protein with a signal mutation, that the wild-type and the *prlA4* mutant form of SecY may act independently in the membrane.

The *SecY* (as well as *secA*) mutants allowed us to identify a novel membrane-spanning intermediate of maltose-binding protein, whose signal peptide has already been removed (C. Ueguchi and K. Ito, manuscript submitted). Pulse-chase experiments using the sec(Ts) mutants at the permissive temperature detected processed molecules that were not releasable to the periplasmic fluid. This species, termed "processed immature form," is in an extended conformation and its maturation into the periplasmic form during the chase was inhibited by energy uncoupler, suggesting that it undergoes a proton gradient-dependent membrane translocation in the absence of covalently attached signal peptide. Based on these obsrvations, we propose that the translocation process can be divided into the early and late phases. The early phase includes targeting of the precursor to the membrane and penetration of the signal peptide plus some 20 residues of the mature sequence, whereas the late phase includes the movement of the bulk of the mature sequence through the membrane. Accumulation of the processed

immature form in the *secY* mutant suggests that SecY function is involved in both the early and the late phases of translocation, only the former being signal sequence-dependent. Mutational and biochemical analysis of the late-phase translocation, as well as characterization of various intermediates in this step, should be extremely important for our understanding of the whole process of protein translocation.

Role of SecY in Membrane Protein Assembly

It has not yet been established how generally the integration of membrane protein is dependent on the *secA* and *secY* genefunctions. Only two cases are known; leader peptidase is *sec* dependent, but M13 coat protein is not (Wolfe *et al.*, 1985). Our studies of the SecY-PhoA fusion proteins indicate that the SecY transmembrane sequences can promote, *secY*$^+$-dependently, the translocation of PhoA (Akiyama and Ito, 1989). In addressing the question of membrane protein assembly, several precautions will be necessary. For instance, there is no reason to assume a uniform mechanism for integration of different proteins, or even different transmembrane segments of a single protein. Likewise, there is no reason to assume a collective "*sec* gene function"; these genes may be differentiated with respect to their involvement in different aspects of protein–membrane interactions. In the case of multi-path membrane proteins, we need assay systems that can tell which transmembrane segment has been inserted in which orientation. Attaching a "reporter" protein such as PhoA enables analysis of local disposition, but does not necessarily reveal the pathway used for the normal intact protein.

We studied *in vivo* integration of the intact lactose permease (LacY), a protein spanning the membrane probably 12 times. Pulse-labeled proteins were assessed for integral membrane anchoring by their resistance to alkali extraction, and LacY protein was identified by immunoprecipitation. LacY in wild-type cells rapidly integrated as shown by its attainment of alkali-resistance soon after pulse-labeling. The integration process was significantly retarded when LacY was synthesized under *secY*-defective conditions or was overproduced from a plasmid (K. Ito and Y. Akiyama, manuscript submitted). Recently, Roepe and Kaback (1989) showed that extensively overproduced LacY protein is extractable by urea and can even be in a water-soluble state upon removal of the chaotrope. Thus, LacY does not spontaneously partition into the membrane. These results suggest that integration of this hydrophobic membrane protein is "catalyzed" by SecY. In contrast, the LacY integration appears less dependent on *secA* and *secB* (K. Ito and Y. Akiyama, unpublished results).

Genetic Suppression of the *secY* Mutations

We isolated temperature-resistant revertants from the *secY24*(Ts) mutant, and studied those revertants which simultaneously gained a cold-sensitive growth phenotype (Shiba *et al.*, 1984b; 1986a). These phenotypes were shown to be due to extragenic suppressor mutations termed *ssy*. The *ssy* cold-sensitive mutations were classified into six classes, *ssyA*, *B*, *D*, *E*, *F*, and *G*, based on their location on the chromosome. Some of them are in genes for protein synthesizing machinery; *ssyF* is in *rpsA* for ribosomal protein S1, and *ssyG* is in *infB* for initiation factor IF2 (Shiba *et al*,. 1986b). Lee and Beckwith (1986), as well as Oliver (1985), obtained similar suppressors from *secA51* and showed that some of them were in protein synthesis factors; even a low concentration of chloramphenicol phenotypically suppressed the *secA* mutant defects (Lee and Beckwith, 1986). These results are generally interpreted to mean that slower translation somehow helps translocation. However, such a notion may not readily explain why the IF2 mutation, which should affect only the initiation reaction, can suppress the export defect caused by *secY24*.

It is intriguing to note that *ssyA* and *ssyB* are most likely to be identical, respectively, with two of the suppressors isolated using Ts *htpR* (*rpoH*) mutant as the primary mutations (K. Shiba, T. Yura, R. Yano, Y. Akiyama, and K. Ito, unpublished results). In this connection, a new mechanism of genetic suppression has recently been revealed. T. K. VanDyk, A. A. Gatenby, and R. A. LaRossa (personal communication) demonstrated that over-expression of the GroES and GroEL proteins, known as the "chaperonine" class of major heat-shock proteins, suppresses a number of temperature-sensitive mutations, including *secY24* and *secA51*. Increased concentration of the GroE proteins may help either the correct formation of protein complex and/or correct folding of polypeptide chains, thereby making the mutated gene products work bette. SecY could form some supramolecular assembly which may either be a stable export machinery or subject to continuous association–dissociation cycles. It is possible that some of the *ssy* mutations are in genes for GroE-like "chaperone" proteins.

Specificity and Interaction of Protein Translocation Factors

As pointed out in the above discussion, it is important to know how different factors for protein export interact. For this end, genetic suppression, crosslinking, and isolation of complexes should be done. Of special interest is whether the two integral membrane factors, SecY and SecE, form complexes in the membrane. Some sequence similarity has been noted between the two proteins (Schatz *et al.*, 1989; note, however, that a low degree of similarity can be found even between SecY and LacY; Ito, 1986).

"Substrate" specificity in the recognition of different factors should also be addressed. From the analyses of mutant phenotypes, there appears to be clear specificity in the cytoplasmic factors that are participating in the "anti-folding" step. Some proteins (OmpA, OmpF, maltose-binding protein, and alkaline phosphatase) are assisted by SecB (Gannon *et al.*, 1989), while others (lipoprotein, ribose-binding protein) are not, and only the plasmid-encoded β-lactamase depends on *groEL* and *groES* (Kusukawa *et al.*, 1989). Membrane-associated factors SecA and SecY appear to be more generally utilized by *E. coli* envelope proteins. Labeling of many proteins recoverable from a periplasmic preparation of the *sec* mutants (Wolfe *et al.*, 1985; Liss and Oliver, 1986) does not necessarily prove that these proteins are independent of the gene functions. Since *sec* mutations usually do not completely arrest the process, one should examine whether a particular precursor accumulates in the cell, rather than whether the mature protein disappears. Although we feel that the SecA-SecY pathway is generally, if not exclusively, used by the secreted (periplasmic and outer membrane) proteins, the question of specificity is entirely open for the cytoplasmic membrane proteins, as already discussed.

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References

Akiyama, Y., and Ito, K. (1985). *EMBO J.* **4**, 3351–3356.
Akiyama, Y., and Ito, K. (1986). *Euro. J. Biochem.* **159**, 263–266.
Akiyama, Y., and Ito, K. (1987). *EMBO J.* **6**, 3465–3470.
Akiyama, Y., and Ito, K. (1989). *J. Biol. Chem.* **264**, 437–442.
Bacallao, R., Crooke, E., Shiba, K., Wickner, W., and Ito, K. (1986). *J. Biol. Chem.* **261**, 12907–12910.
Bankaitis, V. A., and Bassford, P. J. (1985). *J. Bacteriol.* **161**, 169–178.
Berstein, H. D., Pritz, M. A., Strub, K., Hoben, P. J., Brenner, S., and Walter, P. (1989). *Nature (London)* **340**, 482–486.
Bieker, K. L., and Silhavy, T. J. (1989). *Proc, Natl. Acad. Sci. USA* **86**, 968–972.
Blobel, G., and Dobberstein, B. (1975). *J. Cell. Biol.* **67**, 835–851.
Bochkareva, E. S., Lisson, N. M., and Gershovich, A. S. (1988). *Nature (London)* **336**, 254–257.
Brickman, E. R., Oliver, D. B., Garwin, J. L., Kumamoto, C. A., and Beckwith, J. (1984). *Mol. Gen. Genet.* **196**, 24–27.
Cerretti, D. P., Dean, D., Davis, G. R., Bedwell, D. M., and Nomura, M. (1983). *Nucleic Acids Res.* **11**, 2599–2619.
Collier, D. N., Bankaitis, V. A., Weiss, J. B., and Bassford, P. J. (1988). *Cell* **53**, 273–283.

Crooke, E., Brundage, L., Rice, M., and Wickner, W. (1988). *EMBO J.* **7**, 1831–1835.
Deshaies, R. J., Kock, B. D., and Schekman, R. (1988). *Trends Biochem. Sci.* **13**, 384–385.
Emr, S. D., and Bassford, P. J. (1982). *J. Biol. Chem.* **257**, 5852–5860.
Emr, S. D., Hanley-Way, S., and Silhavy, T. J., (1981). *Cell* **23**, 79–88.
Fandl, J. P., and Tai, P. C. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 7448–7452.
Fandl, J. P., Cabelli, R., Oliver, D., and Tai, P. C. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 8953–8957.
Gannon, P. M., Li, P., and Kumamoto, C. A. (1989). *J. Bacteriol.* **171**, 813–818.
Iino, T., and Sako, T. (1988). *J. Biol. Chem.* **263**, 19077–19082.
Inada, T., Court, D. L., Ito, K., and Nakamura, Y. (1989). *J. Bacteriol.* **171**, 585–587.
Ito, K. (1984). *Mol. Gen. Genet.* **197**, 204–208.
Ito, K. (1986). *Adv. Biophys.* **21**, 267–280.
Ito, K., and Akiyama, Y. (1985). *Biochem. Biophy. Res. Commun.* **133**, 214–221.
Ito, K., Wittekind, M., Normura, M., Shiba, K., Yura, T., Miura, A., and Nashimoto, H. (1983). *Cell* **32**, 789–797.
Ito, K., Cerretti, D. P., Nashimoto, H., and Nomura, M. (1984). *EMBO J.* **3**, 2319–2324.
Ito, K., Hirota, Y., and Akiyama, Y. (1989). *J. Bacteriol.* **171**, 1742–1743.
Kusukawa, N., Yura, T., Ueguchi, C., Akiyama, Y., and Ito, K. (1989). *EMBO J.* **8**, 3517–3521.
Lee, C. A., and Beckwith, J. (1986). *J. Bacteriol.* **166**, 878–883.
Lill, R., Cunningham, K., Brundage, L. A., Ito, K., Oliver, D., and Wickner, W. (1989). *EMBO J.* **8**, 961–966.
Lipp, J., and Dobberstein, B. (1986). *Cell* **46**, 1103–1112.
Liss, L. R., and Oliver, D. B. (1986). *J. Biol. Chem.* **261**, 2299–2303.
Manoil, C., and Beckwith, J. (1986). *Science* **223**, 1403–1408.
Muller, J., Reinert, H., and Malke, H. (1989). *J. Bacteriol.* **171**, 2202–2208.
Nomura, M., Gourse, R., and Baughman, G. (1984). *Annu. Rev. Biochem.* **53**, 75–117.
Oliver, D. (1985). *J. Bacteriol.* **161**, 285–291.
Oliver, D., and Beckwith, J. (1981). *Cell* **25**, 765–772.
Oliver, D., and Beckwith, J. (1982). *Cell* **30**, 311–319.
Randall, L. L., and Hardy, S. J. S. (1989). *Science* **243**, 1156–1159.
Riggs, P. D., Derman, A. I., and Beckwith, J. (1988). *Genetics* **118**, 571–579.
Roepe, P. D., and Kaback, H. R. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 6087–6091.
Rosmisch, K., Webb, J., Herz, J., Prehn, S., Frank, R., Vingron, M., and Dobberstein, B. (1989). *Nature* (*London*) **340**, 478–482.
Sako, T., and Iino, T. (1988). *J. Bacteriol.* **170**, 5389–5391.
Schatz, P. J., Riggs, P. D., Jacq, A., Fath, M. J., and Beckwith, J. (1989). *Genes Dev.* **3**, 1035–1044.
Schmid, S. R., and Spiess, M. (1988). *J. Biol. Chem.* **263**, 16886–16891.
Shiba, K., Ito, K., Yura, T., and Cerretti, D. P. (1984a). *EMBO J.* **3**, 631–635.
Shiba, K., Ito, K., and Yura, T. (1984b). *J. Bateriol.* **160**, 696–701.
Shiba, K., Ito, K., and Yura, T. (1986a). *J. Bacteriol.* **166**, 849–858.
Shiba, K., Ito, K., Nakamura, Y., Dondon, J., and Grunberg-Manago, M. (1986b). *EMBO J.* **5**, 3001–3006.
Schultz, J., Silhavy, T., Bermann, M., Fill, N., and Emr, S. D. (1982). *Cell* **31**, 227–235.
Singer, S. J., Maher, P. A., and Yaffe, M. P. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 1015–1019.
Stader, J., Benson, S. A., and Silhavy, T. J. (1986). *J. Biol. Chem.* **261**, 15075–15080.
Ueguchi, C., Wittekind, M., Nomura, M., Akiyama, Y., and Ito, K. (1989). *Mol. Gen. Genet.* **217**, 1–5.
von Heijne, G. (1988). *Biochim. Biopys. Acta* **947**, 307–333.
Wada, A., and Sako, T. (1987). *J. Biochem.* **101**, 1595–1605.
Walter, P., and Lingappa, V. R. *Annu. Rev. Cell. Biol.* **2**, 499–516.
Watanabe, M., and Blobel, G. (1989). *Proc. Natl. Sci. USA* **86**, 1895–1899.
Wolfe, P. B., Rice, M., and Wickner, W. (1985). *J. Biol. Chem.* **260**, 1836–1841.

Stader, J., Benson, S. A., and Silhavy, T. J. (1986). *J. Biol. Chem.* **261**, 15075–15080.
Ueguchi, C., Wittekind, M., Nomura, M., Akiyama, Y., and Ito, K. (1989). *Mol. Gen. Genet.* **217**, 1–5.
von Heijne, G. (1988). *Biochim. Biopys. Acta* **947**, 307–333.
Wada, A., and Sako, T. (1987). *J. Biochem.* **101**, 1595–1605.
Walter, P., and Lingappa, V. R. *Annu. Rev. Cell. Biol.* **2**, 499–516.
Watanabe, M., and Blobel, G. (1989). *Proc. Natl. Sci. USA* **86**, 1895–1899.
Wolfe, P. B., Rice, M., and Wickner, W. (1985). *J. Biol. Chem.* **260**, 1836–1841.