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MINI-REVIEW

SecA Protein: Autoregulated Initiator of Secretory Precursor Protein Translocation Across the *E. coli* Plasma Membrane

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Abstract

Several classes of *secA* mutants have been isolated which reveal the essential role of this gene product for *E. coli* cell envelope protein secretion. SecA-dependent, *in vitro* protein translocation systems have been utilized to show that SecA is an essential, plasma membrane-associated, protein translocation factor, and that SecA's ATPase activity appears to play an essential but as yet undefined role in this process. Cell fractionation studies suggested that SecA protein is in a dynamic state within the cell, occurring in soluble, peripheral, and integral membraneous states. These data have been used to argue that SecA is likely to promote the initial insertion of secretory precursor proteins into the plasma membrane in a manner dependent on ATP hydrolysis. The protein secretion capability of the cell has been shown to translationally regulate *secA* expression with SecA protein serving as an autogenous repressor, although the exact mechanism and purpose of this regulation need to be defined further.

Key Words: SecA; ATPase; protein secretion; protein export; bacterial membranes; gene regulation.

Introduction

The isolation of *secA* mutants in 1980 represented a major advance in the field of bacterial protein export. They were the first mutants isolated which showed pleiotropic secretion defects for periplasmic and outer membrane proteins, implying the existence of at least one essential component for

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envelope protein secretion in *E. coli*. Furthermore, the genetic strategy used in their isolation was generally applicable for obtaining additional secretion-defective (*sec*) mutants. Their discovery came at a time when there was considerable controversy as to whether protein insertion into and translocation across biological membranes was solely self-catalyzed [membrane-trigger hypothesis (Wickner, 1979)] or required a more elaborate export machinery [signal hypothesis (Blobel and Sabatini, 1971)].

The purpose of this study is to (1) critically review what is known about SecA biology, (2) communicate recent data from our laboratory and other laboratories on this subject which are not yet published, and (3) speculate on SecA function in order to stimulate further experimentation. Unfortunately, nearly a decade after the isolation and characterization of the first *secA* mutants, we are not yet in a position to assign a specific role to SecA in promoting cellular protein export. The beginning of our speculation starts with the title of this article, which is our best guess as to at least one of the roles of SecA protein in promoting protein export. Elaboration on this speculation is contained throughout this article, but particularly in the section on the co-insertion model.

Genetic Selections for *secA* Mutants

secA*(Ts) Mutants*

The *secA*(Ts) mutants were isolated by a genetic selection which relied on the principle that a hybrid protein consisting of maltose-binding protein and β-galactosidase, which was normally targeted to the cytoplasmic membrane where it conferred a Lac^- phenotype, could be internalized into the cytoplasm due to a secretion defect, thereby conferring a Lac^+ phenotype. Since a complete secretion defect would be lethal to this cell, *secA* mutants were isolated at 30°C that were partially defective for protein export. However, among such mutants, approximately one-third were found to be temperature-sensitive for growth at 37 or 42°C and exhibited severe protein export defects at the nonpermissive temperatures (Oliver and Beckwith, 1981). The fact that *secA*(Ts) mutations were recessive to wild-type was used to argue that the Ts phenotype was due to a loss of function at the nonpermissive temperature, rather than the acquisition of some new function which would interfere with protein export (and would be expected to be dominant). This argument was important in implying a direct role of the *secA* gene product in protein export prior to a direct biochemical demonstration of this fact (see below). DNA sequence analysis of these mutations and other *secA* mutants described below is given in Fig. 1. Of nine independent *secA*(Ts)

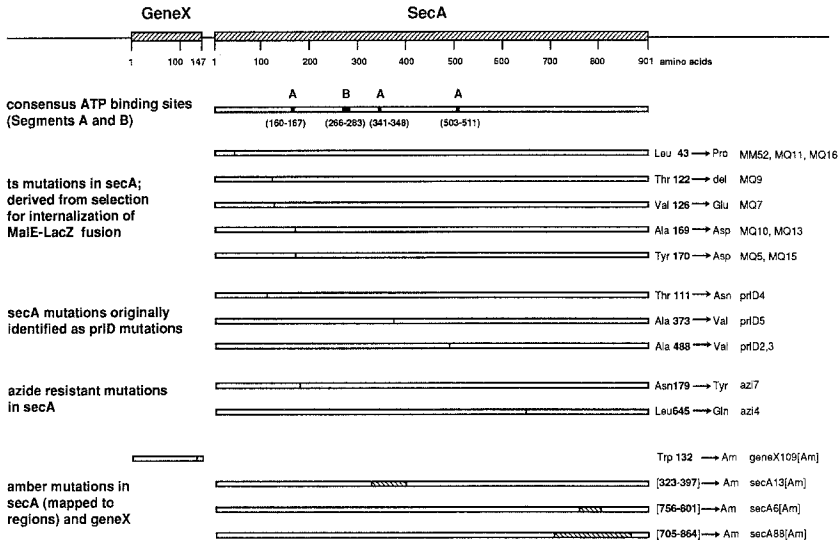

Fig. 1. Summary of *secA* mutations. Predicted ATP-binding (A) and hydrophobic (B) elements were located according to the consensus sequences proposed by Chin *et al.* (1988). GeneX and *secA* are schematized as horizontal bars with vertical lines showing the positions of known mutations within these genes. The amino acid residue number(s) and alteration for a given mutation are indicated at the right, along with the appropriate strain or allele number. Am indicates amber.

mutations analysed, five different alterations were found; all resulted in single amino acid residue alterations within the first 170 amino acid residues of the 901 amino-acid-residue SecA protein (Schmidt *et al.*, 1988). These alterations are predicted to either alter local protein secondary structure or result in a major change in the hydrophobic-hydrophilic character of a given amino acid residue. It is worth pointing out that the mutations corresponding to amino acid residues 169 and 170 are adjacent to one of the predicted ATP-binding sites within the SecA protein (see below). Since the ultimate biophysical basis of the positioning of temperature-sensitive mutations within a protein is not known, it is unclear whether the clustering of temperature-sensitive mutations within the amino-terminus of SecA implies a special role for this region of the protein in export, or is merely due to constraints on protein structure required for a temperature-sensitive phenotype. DNA sequence analysis of the nonconditional *secA* mutants derived from this selection should shed light on this point.

secA(Am) *Mutants*

The *secA(Am)* mutants were isolated using localized mutagenesis techniques and a previously described genetic screening method for detecting

amber mutations in essential genes (Brown *et al.*, 1981). These mutations were introduced into a strain containing a temperature-sensitive amber suppressor in order to be able to conditionally prevent SecA synthesis. The approximate positions of the three known *secA*(Am) alleles are given in Fig. 1. Of the three mutants, only *secA6*(Am) made a substantial amount of a stable amber peptide fragment when shifted to the nonpermissive temperature (37 or 42°C). The severity of the protein export blocks seen when these mutants were shifted to the nonpermissive temperature correlated roughly with the position of the amber mutation within the *secA* gene: the *secA*13(Am) mutant showed the most rapid and severe defects, followed by the *secA6*(Am) mutant, followed by the *secA88*(Am) mutant (Rollo and Oliver, unpublished results). It is worth noting that DNA sequence analysis revealed that an amber mutation originally inferred to be within *secA*, *secA109*(Am), was found to be in the gene immediately upstream of *secA*, gene X, and to be strongly polar on *secA* expression when not suppressed (Schmidt *et al.*, 1988; Schmidt and Oliver, 1989; see *The secA Operon* section below for further explanation).

prlD *Alleles of* secA

One of the other major genetic selections to identify components of the export machinery relies on the isolation of extragenic suppressor mutations that restore the export of proteins with a defective signal peptide (Emr *et al.*, 1981). Such mutations affecting protein localization (*prl*) should in principle be in components of the export machinery which either interact with the signal peptide or bypass the requirement for stringent signal peptide recognition. Extragenic suppressors of a deletion mutation within the signal peptide region of the gene encoding the maltose-binding protein resulted in a class of mutations designated *prlD* which map in the 2 to 3-min region of the *E. coli* chromosome (Bankaitis and Bassford, 1985; Ryan and Basford, 1985; Fikes and Bassford, 1989). Several lines of evidence have been presented indicating that *prlD1* is not allelic to *secA* and represents an as yet uncharacterized *sec* gene (Bankaitis and Bassford, 1985). However, the *prlD2*, *prlD3*, *prlD4*, and *prlD5* mutations have been shown to be alleles of *secA* (Fikes and Bassford, 1989), and this information is given in Fig. 1. It should be cautioned, however, that the existence of these mutations in *secA* does not necessarily imply that this protein directly interacts with signal peptides. As Randall *et al.* (1987) have correctly pointed out, a genetic argument for protein–protein interaction relies on allele-specific effects of appropriately constructed double mutant strains on the process under study, and such effects have not been demonstrated. However, recent biochemical evidence presented below suggests that SecA protein does interact directly with the signal peptide of secretory precursor proteins. Until biochemical and

biophysical data are available indicating which regions of SecA are involved in these interactions, speculation as to the location(s) of these contact sites is premature. Certainly these mutations could cause three-dimensional changes in the configuration of the SecA protein which would expose a signal peptide binding region(s) distant from the sites of these alterations.

Azide-Resistant Alleles of secA

The sodium azide-resistant mutations are among the oldest known mutations in *E. coli* (Lederberg, 1950). Earlier studies indicated that sodium azide resistance (*azi*) and phenethyl alcohol resistance (*pea*) were probably allelic, since both types of mutations were tightly linked around 2.5 min on the *E. coli* chromosome and selection for one type of resistance often altered the other type of resistance (Yura and Wada, 1968). It had also been demonstrated previously that phenethyl alcohol addition to *E. coli* cultures resulted in a defect in protein export as indicated by secretory precursor protein accumulation (Tribhuvan *et al.*, 1970). Based on these observations and a suggestion by Barbara Bachmann, our laboratory has analyzed four different sodium azide-resistant mutations and found them to be allelic to *secA* (Oliver, Dolan, Cabelli, and Jarosik, manuscript in preparation). Genetic mapping allowed the assignment of these mutations into two narrow regions within *secA*, and DNA sequence analysis of a representative mutation from each region showed that they resulted in the single amino acid residue alterations shown in Fig. 1. It is worth noting that *azi7* is adjacent to one of the predicted ATP binding sites within SecA (see below).

A direct inhibition of SecA protein's translocation activity by sodium azide was indicated by the fact that addition of 1 mM sodium azide to a wild-type *E. coli* culture resulted in a nearly complete block in protein export after 1 min, and that isogenic *azi* mutants were completely resistant to such inhibition. Dominance and recessive studies indicated that *azi* mutations were codominant with wild-type in that most of the SecA produced by the cell had to be of the sodium azide-resistant form in order for the cell to be resistant to sodium azide inhibition. This implies that the sodium azide-inhibited SecA protein must be titrating out some cellular component essential for protein export.

The origin of sodium azide-resistant mutations within the *secA* gene raises some intriguing questions concerning the effect of sodium azide on *E. coli* cell biology. First, why is SecA protein normally a target for sodium azide inhibition and what kind of alterations in SecA protein are needed to confer resistance? Second, why do mutations in the *secA* gene alone confer resistance of the cell to sodium azide? In answer to the first question, it appears likely that SecA ATPase activity (explained below) is the target for

sodium azide inhibition, since ATPases are often targets for sodium azide inhibition. One mechanism of azide inhibition of ATPase activity appears to involve a binding/modification of a site on the enzyme which prevents an allosteric change needed for an increase in the catalytic rate (Noumi *et al.*, 1987). In this kind of scenario, the *azi* alleles of *secA* could represent the loss of a sodium azide binding/modification site on SecA protein or a form of the protein that is locked into an activated state. With regard to the second question, clearly SecA is not the only target of sodium azide inhibition within the cell, since the F1 ATPase is also sensitive to azide inhibition (Kobayashi and Anraku, 1972). However, we presume that, like F1, the other targets of sodium azide inhibition are not needed for anaerobic growth in minimal media due to redundancies in metabolic pathways and essential processes. For additional discussion of the effect of sodium azide on SecA ATPase activity, see below.

Extragenic Suppressors of secA*(Ts) Mutants*

In order to genetically identify other essential cellular components which interact with SecA protein, cold-sensitive extragenic suppressors of the *secA51*(Ts) mutation were isolated (Brickman *et al.*, 1984; Ferro-Novick *et al.*, 1984; Oliver, 1985). Since the conditions under which the suppressors were isolated still allowed partial *secA* function (37°C), this approach tended to select for mutations which depressed the rate of protein translation, thereby putting the processes of secretory protein synthesis and export back into balance. This paradigm was supported by experiments from Lee and Beckwith (1986), who showed that partial inhibition of protein synthesis by genetic (tRNA[Ts] or tRNA synthetase[Ts] mutants) or biochemical means (subinhibitory concentrations of chloramphenicol) suppressed the growth and secretion defect found in the *secA51*(Ts) mutant. Extragenic suppressor mutations were found in the genes encoding ribosomal proteins S15 (cited in Beckwith and Ferro-Novick, 1986) and L34 (Oliver, 1985; Jarosik and Oliver, unpublished data), as well as five unidentified essential genes (Oliver, 1985; Grodberg and Oliver, unpublished results). One of the extragenic suppressor mutations, *ssaD1*, which mapped very near *secD*, has been shown to be distinct from this gene (Gardel *et al.*, 1987). The first extragenic suppressor mutation isolated, that was originally ascribed to the *secY* (*prlA*) gene, in fact is located in a ribosomal protein gene immediately upstream of this gene (Ito, unpublished results). A similar situation has emerged for the isolation and characterization of extragenic suppressor mutations of the *secY24*(*Ts*) mutant (Shiba *et al.*, 1986a,b). Taken together, these studies underscore that secretory protein synthesis and export are balanced processes within the cell, and point to a possible functional coupling between the two

processes which needs to be explored further. Furthermore, such studies suggest that the extragenic suppressor approach to defining functional interactions between secretion machinery components could be productive if the appropriate primary mutant and selective conditions were chosen such that the secretion defect was more absolute under the selective conditions employed.

Export Physiology of *secA* Mutants

The *secA51*(Ts) and *secY24*(Ts) mutants have been widely used by investigators to measure the requirement of a given secretory protein for the *E. coli* export machinery. No case has been observed to our knowledge where a dependence (or independence) on SecA protein for translocation of a given envelope protein has not also been accompanied by a similar dependence (or independence) on SecY protein. A number of cell envelope proteins have been examined for their *secA*-dependence and these data are summarized in Table I. In general, we have not included a number of exported proteins of prokaryotic and eukaryotic origin whose heterologous expression and export has been investigated in *secA* mutants. Three precautionary notes should be emphasized in interpreting these data. First, in many cases the presence of unprocessed, precursor protein detected by pulse labeling of the temperature-shifted *secA*(Ts) mutants with radioactive amino acids has been taken as sufficient evidence for *secA*-dependent export. Since the active site of the major *E. coli* signal peptidase is located on the periplasmic side of the plasma membrane (Wolfe *et al.*, 1983) and processing normally occurs as the polypeptide chain is translocated across the membrane, this criterion for *sec*-dependent export is usually sufficient. Corroborative experiments employing cell fractionation and protease accessibility studies have been performed in certain cases. Second, and more importantly, it should be emphasized that *sec*-dependent export is an operational definition and is, therefore, subject to experimental conditions. Most investigators have measured dependence by pulse labeling techniques with radioactive amino acids after 1–3 hr of growth in minimal media (0.5–3 doublings) at the nonpermissive temperature. It is clear that extensive growth at the nonpermissive temperature resulted in a range of physiological alterations (de Cock *et al.*, 1989), making it difficult to determine whether the export defects observed were due to primary or secondary effects. For example, reports that utilized the secA109(Am) strain indicated that there was a mechanistic coupling between protein synthesis and protein export mediated by SecA protein (Oliver and Beckwith, 1982; Kumamoto *et al.*, 1984). However, it appears that the requirement for SecA protein synthesis for maltose-binding protein synthesis and export was not

Table I. *E. coli* Envelope Protein Biogenesis

Protein	References[a]
I. *secA*-Dependent	
A. Periplasmic proteins	
Alkaline phosphatase	7
Beta-lactamase (TEM)	5
Cysteine-binding protein	5
Leucine-specific-binding protein	5
Maltose-binding protein	5, 7
Ribose-binding protein	3, 5
Xylose-binding protein	5
B. Outer membrane proteins	
Lambda receptor protein	5, 7
Lipoprotein (murein)	4, 5, 8
OmpA	4, 5, 8
OmpF/C	5, 7
TonA	1
C. Inner membrane proteins	
Leader peptidase	8
Tsr chemosensory transducer	2
II. *secA*-Independent	
A. Inner membrane proteins	
Bacteriophage M13 coat protein	8
B. Excreted proteins	
Hemolysin	6

[a] 1. Baker *et al.*, 1987; 2. Gerbert *et al.*, 1988; 3. Garwin and Beckwith, 1982; 4. Hayashi and Wu, 1985; 5. Liss and Oliver, 1986; 6. Mackman *et al.*, 1987; 7. Oliver and Beckwith, 1981; 8. Wolfe *et al.*, 1985.

only due to a deficiency in SecA protein, but also to an unknown defect in the catabolite activator system which resulted in poor synthesis of maltose-binding protein (Strauch *et al.*, 1986). Third, our laboratory carefully examined the export blocks found in *secA*, *secY*, and *secD* mutants grown at the nonpermissive temperature for 1–3 hr using the standard pulse-chase radiolabeling methodology, and came to the conclusion that the available *sec* mutants were leaky for the export block (Rollo and Oliver, unpublished results). Most precursor proteins visualized by a 1-min pulse-label displayed a slow and semiquantitative processing during the course of a 30-min chase period. This pattern is most easily explained by assuming that the available *sec* mutants are probably temperature sensitive for synthesis and therefore the residual export capacity is dependent on the extent that export machinery made prior to the temperature shift is diluted out by subsequent growth at

the nonpermissive temperature. Given these findings, it is likely that if a given envelope protein requires only 10% as much active SecA protein as most envelope proteins do, it would be scored as *secA*-independent for translocation. Clearly, better *sec* mutants and inhibitors of the protein export machinery (see sodium azide above) will be required before these issues can be resolved.

Several general patterns of *secA*-dependent export are evident from the data presented in Table I. SecA is required for the export of most *E. coli* periplasmic and outer membrane proteins examined. Integral membrane proteins which possess a cleaved or uncleaved signal peptide and a substantial exported segment also show *secA*-dependence for translocation of the exported segment. The rules regarding the *secA*-dependence of integral membrane proteins which do not possess a signal peptide and exported segment of any appreciable size remain unclear; this family of proteins needs to be studied further to clarify such requirements. An experiment from Baker *et al.* (1987) indicated that the rates of incorporation of newly synthesized bulk protein into inner and outer membrane were depressed similarly in the *secA*(Ts) mutant grown at the nonpermissive temperature.

Two types of *secA*-independent export have been inferred. One case, typified by small integral membrane proteins possessing a signal peptide (generally less than 75 amino acid residues), such as M13 procoat, not only has been shown to be *secA* and *secY*-independent for export in *E. coli*, but also has been shown to be SRP and SRP-receptor independent for their insertion into mammalian microsomal membranes (Watts *et al.*, 1983; Cobet *et al*, 1989). However, in this instance procoat insertion does display a requirement for ATP hydrolysis, a protein of the Hsp70 family, and an as yet unidentified proteinaceous component of the reticulocyte lysate (Wiech *et al.*, 1987; Zimmerman *et al.*, 1988). It is tempting to speculate that this ATP-dependent export shares the same mechanistic details as ATP-dependent export in *E. coli*. If this is the case, then the unidentified component in this system may serve a homologous function to SecA protein (see characterization of *SecA ATPase* below). Genetic engineering techniques have been used to create derivatives of M13 procoat which contain additional amino acid residues in regions of the protein which traverse the plasma membrane; such hybrid proteins are now dependent on *secA* and *secY* for their membrane insertion (Kuhn, 1988). It appears likely that it is the length and complexity (e.g., charge distribution) of the protein segment that is translocated into or through the plasma membrane which determines its dependence on the bacterial protein export machinery. The second case of *secA*-independent protein export is typified by more exotic proteins such as haemolysins, which are excreted into the extracellular medium. Protein export in these cases requires accessory proteins which appear to be specific

for a given secretory protein. Here again, there may be a SecA analog in the best studied of these cases, since a predicted membrane-bound ATPase (HlyB) is required for the translocation of haemolysin across the plasma membrane (Gerlach *et al.*, 1986).

Regulation of *secA* Expression

Derepressing Signals

It has been found that the synthesis of SecA protein appears to be responsive to the protein secretion capability of the *E. coli* cell. When protein export is blocked, SecA protein synthesis rises 10- to 20-fold. This derepression in SecA synthesis has been found for secretion blocks in *secA*, *secD*, *secE*, and *secY* mutants, but not the *secB* null mutant (Riggs *et al.*, 1988; Rollo and Oliver, 1988). SecA levels were also elevated by secretion blocks imposed by a high level of synthesis of an export-defective, MalE-LacZ hybrid protein (Oliver and Beckwith, 1982). The lack of derepression of SecA synthesis found in the *secB* null mutant did not appear to be due to a requirement for SecB in *SecA* regulation, but rather the failure to generate the proper derepressing signal. This conclusion was supported by the fact that *secY*(Ts) *secB* null, double mutants did derepress *secA* expression when grown at the nonpermissive temperature (Rollo and Oliver, 1988). Inhibition of the *secA*-dependent export by sodium azide also caused elevation of SecA protein synthesis (Oliver, Dolan, Cabelli, and Jarosik, manuscript in preparation).

The secA *Operon*

A large region of DNA encompassing the *secA* gene has been sequenced (Beall and Lutkenhaus, 1987; Schmidt *et al.*, 1988; Akiyama *et al.*, 1987), and the results are summarized in Fig. 2. The *secA* gene is found around 2.5 min on the *E. coli* chromosome, downstream of a well-characterized region encoding proteins involved in peptidoglycan synthesis, cell division, and cell envelope biogenesis (Lutkenhaus *et al.*, 1980). Immediately downstream of a rho-independent transcriptional terminator at the end of the *envA* gene, there

Fig. 2. Organization of the *secA* operon. Promoters (P), transcriptional direction (arrows), transcriptional terminators (t), and transcriptional read-through (dotted arrow) are indicated. The genes are shown blackened with the marker bar representing 1 kb of genetic distance.

lies a three-gene operon consisting of geneX (a gene of unknown function), *secA*, and *mutT* [a gene involved in the fidelity of DNA replication (Bhatnagar and Bessman, 1988)]. At the end of this operon there is a set of extragenic palindromic repeated sequences which have been found at the end of several bacterial operons (Watson, 1985). That these three genes in fact constitute an operon is supported by several lines of evidence. First, it was shown that geneX109(Am) or insertion mutations were strongly polar on *secA* expression (Schmidt *et al.*, 1988; Fikes and Bassford, 1989). Second, this three-gene operon was cloned and *lacZ* protein fusions were constructed in each cistron. Deletion of the presumed promoter sequence upstream of geneX abolished expression of each of these fusions (Schmidt and Oliver, manuscript submitted). Third, transcriptional mapping studies indicated that *secA* expression was derived from two transcripts of approximately equal abundance: one appeared to be a read-through transcript from *envA* and the other originated at the putative promoter sequence upstream from geneX (Schmidt and Oliver, 1989). Whether there is a functional significance to combining these genes into an operon is unclear at the present moment and certainly must await the discovery of a function for geneX. One unusual feature of this operon is the different levels of expression of the three gene products. The *lacZ* fusion analysis cited above indicated that MutT levels are approximately 10% of SecA levels, presumably due to the presence of a putative rho-independent transcriptional terminator located between *secA* and *mutT*. GeneX levels are only about 1% of SecA levels, presumably due to the absence of any recognizable ribosome-binding site upstream of this gene. It is also of interest in this regard that immediately upstream and downstream of the *secA* translational initiator, there is a sequence with a strong homology to a translational enhancer sequence that has been described recently for abundantly expressed genes often contained in bacteriophage genomes (Olins and Rangwala, 1989).

Autogenous Translational Control

The derepression of *secA* expression by protein export defects has been investigated and shown to be due to regulation at the translational level. Two lines of evidence support this contention. First, measurement of geneX-*secA* mRNA levels before and after an export block revealed no difference in the level of this transcript despite greater than 10-fold differences in the level of SecA protein synthesis (Schmidt and Oliver, 1989). Second, *secA-lacZ* protein fusions but not *secA-lacZ* operon fusions were derepressed by protein export defects (Schmidt and Oliver, 1989). This analysis has been extended recently to geneX, and indicated that geneX-*lacZ* protein fusions were not derepressed by protein export defects (Schmidt and Oliver, manuscript submitted). These additional data argue that the regulated site is around the translational initiation region for *secA*. This presumption has been supported by showing that

a deletion which removed the region between the termination codon of geneX and the ribosome-binding site of *secA* rendered *secA* translation constitutively high under all export conditions tested (Schmidt and Oliver, manuscript submitted). Thus it would appear that *secA*'s ribosome-binding site region is somewhat masked during normal protein secretion and becomes unmasked during conditions of protein export blockage. It is of interest that *secA* translation appears to be coupled to geneX, since the geneX109(Am) mutation, which prematurely terminates geneX translation 16 codons before the normal stop, was completely polar on secA expression; this observation was unlikely to be due to transcriptional polarity (Schmidt and Oliver, 1989). There is evidence of a similar nature for another *sec* gene, in that *secY* translation is apparently coupled to *rpoO* translation (Akiyama and Ito, 1987). It is worth pointing out that the data on translational regulation of *secA* still allow for the possibility of a modest level (severalfold) of transcriptional regulation of *secA* expression (Schmidt and Oliver, 1989).

A strain has been constructed which demonstrated that SecA is an autogenous repressor of its own synthesis during normal protein export conditions (Schmidt and Oliver, 1989). In this strain, overproduction of SecA (approximately 10-fold) from a geneX-*secA*-containing plasmid was responsible for super-repression of a chromosomally encoded, *secA-lacZ* protein fusion. The SecA51(Ts) protein was only 50% as active in repression as wild-type SecA in this assay. Whether overproduction of GeneX protein is also required for the observed repression has not yet been addressed. Since repression of the *secA-lacZ* reporter by the plasmid encoded geneX-*secA* operon can easily be detected colorimetrically on lactose tetrazolium plates, our laboratory has recently used two-codon, linker mutagenesis of this plasmid in an attempt to define regions of *secA* which are involved in autogenous repression (Jarosik and Oliver, manuscript in preparation). A large collection of linker-mutagenized plasmids was used to transform the *secA-lacZ* fusion-containing strain, and colonies which showed a fully Lac^+ (derepressed) phenotype were picked and characterized. Approximately 90% of linker insertions were found within two regions of the *secA* gene (corresponding to amino acid residues 179–252 and 785–809). To our surprise, when physiological studies of these strains were performed, we discovered that this approach had yielded dominant alleles of *secA*-encoding proteins defective in SecA-dependent translocation activity. Obviously, the production of forms of SecA which interfere with protein export would also result in derepression of the *secA-lacZ* reporter. Since a large number of randomly inserted, linker mutations were screened in this study, and no mutants were found which clearly separated SecA's autogenous repressor activity from its translocation activity, we concluded that these activities must be heavily overlapping at the level of the structure of the SecA protein.

Mechanism and Purpose of secA *Regulation*

From the results presented above, it appears that *secA* translation is kept at a lower level during normal protein export, and that *SecA* protein is somehow required to effect this repression. An obvious model to explain these data would involve SecA protein directly binding to its own mRNA in the translational initiation region, thereby somewhat masking the ribosome-binding site either directly or through the formation of an appropriate RNA secondary structure. The indication that *secA* is translationally coupled to geneX lends support to the notion of a partially "closed" translation initiation region for *secA*. A variation of this model would be to have SecA protein communicate with a target protein, which would be the RNA-binding protein and ultimate repressor. While other models involving regulation at the level of translational elongation or functional decay of *secA* mRNA cannot be excluded at this time, the regulated site would have to be before the middle of the *secA* gene (because of the *secA-lacZ* fusion employed in the studies cited), and such models are not favored at this time.

It is unclear at this point what the derepressing signal for *secA* expression is, and how it is triggered by most types of protein export defects. The secretion defects which do derepress *secA* (*secA*, *secD*, *secE*, and *secY* mutants and secretion-defective hybrid proteins containing a β-galactosidase moiety) all have the common theme that they result in secretion defects at the level of the membrane [secA is a peripheral membrane component (see below), SecD, SecE, and SecY are or have been inferred to be integral membrane components (Akiyama and Ito, 1987; Schatz *et al.*, 1989; Jon Beckwith, personal communication)]. The one secretion defect which does not derepress *secA* (the *secB* null mutant) is the result of a secretion defect in a cytoplasmic translocation factor (Kumamoto *et al.*, 1989; Watanabe and Blobel, 1989a,b). If SecA protein is responsible for delivering membrane-associated, secretory precursor proteins to the more integral membranous, Sec machinery, then defects in any one of these components, including SecA itself, could prevent proper transfer. Such transfer could also be defective for fusion proteins such as the MalE-LacZ hyrid proteins. Therefore, all of the membrane-associated secretion defects would have a common effect, namely the buildup of SecA-secretory precursor protein complexes blocking SecA repressor function. In this line of thinking, the available pool of free SecA (either cytoplasmic or membrane-associated) would determine the level of *secA* repression observed under a given export condition. In an alternative model, the translocation reaction itself would create a transient modification of SecA protein (e.g., phosphorylation) which would be necessary for SecA repressor activity. This post-translational modification would, therefore, not occur during a block in the translocation

cycle. In this regard, it is notable that the size of the cytoplasmic SecA pool varies significantly from the *secA51*(Ts) and *secY24*(Ts) mutants and in the strain overproducing the MalE-LacZ hybrid protein, and that attempts to separate *secA* regulation from SecA translocation activity have been unsuccessful (Cabelli, Dolan, and Oliver, manuscript in preparation; Jarosik and Oliver, manuscript in preparation). Both of these results favor the second type of model.

The ultimate purpose of *secA* regulation is not known. There is no evidence to date that we are aware of indicating that other Sec components are regulated similarly to SecA. This unique position among the *sec* genes may have to do with SecA occupying a pivotal role in the protein export pathway that is rate-limiting for the overall process. If SecA is the initiator of secretory precursor protein insertion into the membrane (beyond the initial signal peptide insertion into the membrane; see below), then this may be a mechanistically slow step which is particularly sensitive to substrate excess. Alternatively, SecA limitation may be necessary so as not to overload the integral-membrane, Sec machinery with secretory precursors which could transiently remain in the cytoplasm complexed with various soluble chaperones. Finally, with the discovery that SecA ATPase activity can be activated by phospholipids in the absence of secretory precursor proteins under certain conditions (see below), SecA limitation may have evolved as a mechanism to prevent ATP wastage.

SecA Subcellular Localization

SecA was originally described as a peripheral membrane protein whose extent of membrane association depended on the fractionation conditions employed (Oiver and Beckwith, 1982). Our laboratory has studied the subcellular localization of SecA protein more thoroughly with particular emphasis on determining whether SecA protein interacts with any other soluble or membrane-associated component(s). This analysis has been carried out by lysis of *E. coli* cells in the french pressure cell, followed by fractionation of components by gel filtration using superose 6 FPLC chromatography and by velocity and density sucrose gradient sedimentation followed by analysis of SecA protein and other markers by immunoblotting techniques. The most pertinent findings of these studies (Cabelli, Dolan, and Oliver, manuscript in preparation) are summarized in Table II. We found two major, equally abundant, forms of cellular SecA protein: a cytoplasmic form (form I) that appeared monomeric in size by sedimentation analysis, and a membrane-bound form(s) (form II) that was largely associated with the cytoplasmic membrane. While the majority of membrane-bound SecA was peripheral in

Table. II. Forms of SecA

Form of SecA	Properties
Form I	Soluble. Sediments as expected for a 102 kDa protein monomer.
Form II	Soluble. Sedimentation and gel filtration suggest an M_r of 400–1000 kDa. Extremely labile. Amount may be increased under conditions of protein export block.
Form IIIa	Insensitive to DNase. Associated with inner membrane. Lability to membrane perturbants suggests a peripheral association.
Form IIIb	Insensitive to DNase and RNase. Associated with inner membrane. Lability to membrane perturbants suggests an integral orientation within the membrane.

nature (form IIIa), a substantial fraction (approximately 20–30%; form IIIb) was not removed by extraction with 0.2 N sodium hydroxide, 1 M hydroxylamine, or 1.5 M urea, and therefore was more integral in nature (Chen and Tai, personal communication; Cabelli and Oliver, manuscript in preparation). We have interpreted this latter form of SecA as one that was inserted more deeply into the membrane, perhaps in an export and precursor protein-dependent fashion (see below for additional data). A minor, but-high-molecular weight, cytoplasmic form of SecA detected (form II) could have been a SecA multimer or a complex of SecA and another protein(s), but this form was labile and was not purified further. We currently do not have any evidence supporting SecA–ribosome or SecA–polysome interactions, nor have we been able to detect any soluble complexes between wild-type SecA protein and any secretory precursor proteins. However, we have not yet examined enough conditions to rule out these types of interactions. Since it is clear that membrane-bound SecA is sufficient to catalyze protein translocation *in vitro* (see below), it could be argued that the cytoplasmic forms of SecA detected in these studies represented artifacts of the fractionation procedures employed. However, immunoelectron microscopy of thin sections of wild-type *E. coli* cells showed that the apparent cytoplasmic and membrane distribution of SecA protein was similar to that given in Table II. In addition, there appeared to be an enrichment for SecA protein at adhesion sites between inner and outer membranes (Bayer, Cabelli, and Oliver, unpublished results).

A similar analysis on the distribution of SecA protein within the cell has been conducted on the *secA*(Ts) mutant with most interesting results. In this mutant approximately 90% of the SecA(Ts) protein was associated with the membrane fraction. Two possibilities were considered: either the presence of the export defect in this strain held the SecA51(Ts) protein on the membrane due to association with secretory precursor proteins, or the SecA51(Ts) protein had an inherently greater affinity for the plasma membrane. That the

latter possibility was the correct one was supported by an experiment where two copies of the *secA* gene were introduced into a strain: one encoding an electrophoretically variant, but export-proficient form of SecA, and one encoding the SecA51(Ts) protein. Subcellular fractionation studies of the resulting export-proficient strain showed that the SecA51(Ts) protein was still approximately 90% membrane-bound, while the electophoretically variant SecA was approximately 50% membrane-bound as in a wild-type strain (Cabelli, Dolan, and Oliver, manuscript in preparation).

Since the studies described above, as well as those to be described below, indicate that SecA association with the plasma membrane is a critical part of its biological function, our laboratory has attempted to determine whether one particular region of SecA protein is responsible for membrane binding. This analysis employed a nested set of *secA-lacZ* fusions encoding an increasingly larger portion of SecA protein fused to a constant amount of enzymatically active, β-galactosidase (Cabelli, Dolan, and Oliver, manuscript in preparation). SecA-LacZ fusion proteins containing as little as 31 and 67 24amino-terminal, amino acid residues of SecA showed considerable membrane binding character (72 and 54% membrane association, respectively, versus $< 1\%$ for β-galactosidase alone). This result may be due to the presence of a predicted amphipathic α-helix early in the SecA protein. SecA-LacZ fusion proteins containing 239 and 324 amino-terminal, amino acid residues of SecA were completely membrane-bound ($> 98\%$), whereas a larger fusion protein containing 422 amino-terminal, amino acid residues of SecA displayed a similar pattern to wild-type SecA protein in its association with the membrane (62 and 39%, respectively). These data suggest that there may be several membrane-binding (or stabilization) elements within the first 250 amino-terminal, amino acid residues of SecA protein, and that more distal regions of the protein may facilitate a release step which keeps the protein in some type of dynamic equilibrium regarding membrane association. It is currently unknown whether SecA protein utilizes a proteinaceous receptor for membrane attachment, although the purified protein is capable of binding to and inserting into liposomes which contain acidic phospholipids (Lill *et al.*, 1990; London, Cabelli, and Oliver, unpublished results). For further discussion of this point, see below.

Localization studies which employ hybrid proteins must be regarded with caution, since gene fusion techniques can result in the generation of abnormal regions of protein structure which create artifacts in the biological behavior of these proteins. In this regard, however, we have discovered recently that strains containing a plasmid encoding the 239 amino-terminal amino acid residues of SecA protein produced an appropriate-sized SecA peptide that bound quantitatively to the membrane. In this location the SecA peptide possessed biological activity since it complemented the growth and

secretion defect of the *secA51*(Ts) mutant (Cabelli, Dolan, and Oliver, manuscript in preparation). That both the SecA51(Ts) protein and this peptide fragment of SecA participate together in protein translocation was indicated by the fact that production of this SecA fragment did not complement the defects found in the *secA13*(Am) mutant. These important findings raise the possibility that SecA promotes the protein translocation reaction in the membrane as a multimer, or that SecA has at least two separable activities.

In Vitro Function of SecA Protein

SecA-Dependent In Vitro *Translocation Systems*

Two different SecA-dependent *in vitro* protein translocation systems have been constructed. One system utilizes the basic *in vitro* translocation system developed by Tai and coworkers (Chen and Tai, 1985, 1987) in which secretory precursor proteins are synthesized *in vitro* and are delivered to inverted plasma membrane vesicles either co-translationally or post-translationally. This system was made SecA-dependent by utilizing the *secA13*(Am) mutant grown under nonsuppressing conditions to produce cells depleted of SecA protein which were then used to prepare S30 extracts and inverted plasma membrane vesicles (Cabelli *et al.*, 1988). Pivotal to this work was the construction of a SecA-overproducing strain and the purification of SecA protein to homogeneity. This allowed a direct demonstration that SecA depletion from components of the *in vitro* system resulted in a loss of translocation for proOmpA and alkaline phosphatase precursor, and that addition of purified SecA protein to the depleted system restored precursor translocation. Biochemical complementation of this defect was demonstated whether these two precursor proteins were presented to the system co-translationally or post-translationally. This work also showed that SecA protein was fulfilling a membrane-associated function in this *in vitro* translocation system. Reconstitution of SecA-depleted inverted plasma membrane vesicles with SecA protein restored protein translocation activity in a system otherwise lacking any additional SecA protein.

A second and simpler, SecA-dependent *in vitro* translocation system was developed by Wickner and coworkers (Cunningham *et al.*, 1989). In this system a purified secretory precursor protein (such as proOmpA or prePhoE) was kept in an export-competent state by solublization in urea and was translocated *in vitro* by dilution into a suspension of inverted plasma membrane vesicles. The vesicles were made SecA-dependent by treatment with urea which removes/inactivates endogenous SecA protein (see above for

further explanation). This system was used to demonstrate that there was an obligate order of binding of precursor and SecA proteins to inverted plasma membrane vesicles for productive translocation to occur: membrane binding of SecA protein prior to binding of the secretory precursor protein was necessary for translocation to occur. This conclusion must be qualified, however, by the fact that this was a highly purified system lacking additional soluble and peripheral membrane factors which could stabilize membrane-associated precursor proteins prior to their association with SecA protein.

Recently this latter SecA-dependent *in vitro* translocation system has been used to demonstrate that the presence of acidic phospholipids in the inverted plasma membrane vesicles was necessary for functional binding of SecA protein to these vesicles (Lill *et al.*, 1990). In this study a strain was employed in which the content of acidic phospholipids (phosphatidylglycerol and cardiolipin) within the membrane was reduced in a conditional and highly specific manner to approximately 15% of the wild-type level (Heacock and Dowhan, 1989). Inverted plasma membrane vesicles prepared from this strain were largely defective in SecA protein binding and *in vitro* protein translocation. This result is in accord with an earlier study which demonstrated a requirement for acidic phospholipids to promote rapid and efficient protein export *in vitro* and *in vitro* (deVrije *et al.*, 1988). Further studies showed that purified SecA protein only bound to liposomes which contained acidic phospholipids (Lill *et al.*, 1990; London, Cabelli, and Oliver, unpublished results). We have recently found that such binding corresponded to insertion of SecA protein into the membrane. In these studies, phospholipids with bromines at C9 and C10 positions on the fatty acyl chain were used to make liposomes, and bromine quenching of SecA protein's tryptophan fluorescence was used to monitor SecA insertion into liposomes. By comparing liposomes with and without brominated phospholipids, it was concluded that tryptophan residues in SecA insert into the lipid bilayer (London, Cabelli, and Oliver, unpublished results).

The binding and reconstitution studies cited above raise the question of whether SecA also possesses a proteinaceous membrane receptor. While the answer to this is unknown, the localization studies cited above are compatible with this notion. A low-affinity form of SecA (form IIIa, see Table II) would require acidic phospholipids for association with the membrane, and a higher-affinity form (form IIIb) could be the result of insertion into the membrane and/or association with other integral membrane Sec-machinery components. There are several reports suggesting an interaction of SecA protein with SecY protein. Tai and coworkers demonstrated a *secY24*(Ts) defect for *in vitro* protein translocation by prior *in vitro* heat inactivation of inverted plasma membrane vesicles prepared from this strain (Fandl and Tai, 1987). Interestingly, addition of purified SecA protein to this translocation-

incompetent system restored translocation of proOmpA and alkaline phosphatase precursor (Fandl *et al.*, 1988). If the *secY24*(Ts) defect represented a form of SecY protein less able to bind SecA protein, then the presence of additional SecA protein could compensate for this defect. A second study relates to the requirement of SecY protein to activate SecA ATPase activity (explained below). Here it was shown that inverted plasma membrane vesicles prepared from the *secY24*(Ts) mutant grown at 42°C or prepared from the wild-type strain but preincubated with SecY antibody are incapable of activating SecA ATPase activity (Lill, *et al.*, 1989). While both of these studies are suggestive of a physical interaction of SecA protein with SecY protein, they clearly stop short of direct proof. Furthermore, they leave unanswered the question of the ultimate purpose of this putative interaction. Additional studies will be required to resolve these issues.

The functional binding of SecA protein to inverted plasma membrane vesicles may also be facilitated by an energized membrane. A recent study indicated that a protonmotive force was required for the translocation of certain precursor proteins into plasma membrane vesicles (Yamada *et al.*, 1989a), and that supplementation of the *in vitro* system with additional SecA protein bypassed this requirement (Yamada *et al.*, 1989b). The effect of a protonmotive force on SecA binding to these membranes was not explored. It is unclear, therefore, whether the protonmotive force was having an effect on SecA binding to the membrane or its interaction with other integral membrane Sec proteins. Presumably the protonmotive force could affect the conformation of SecA protein, other membrane-associated, Sec machinery components, or the precursor protein itself within the membrane environment.

Both of the SecA-dependent *in vitro* protein translocation systems currently in use should prove invaluable in dissecting SecA's role(s) in promoting presecretory protein translocation across the plasma membrane. Clearly the stage is set to tackle this major question. However, caution must be exercised in assuming that these *in vitro* translocation systems accurately mimic all the steps which *in vivo* protein export normally encompasses. Although we are unsure as to whether soluble SecA, free or in some protein complex, plays a role in protein secretion, there are legitimate grounds to question whether either *in vitro* system would reveal such an activity. The enrichment for SecA protein at adhesion sites between inner and outer membranes (see above) points to a level of organization which is lost in the present *in vitro* translocation systems. Clearly, future effort is warranted to continue to undertake *in vivo* studies which would uncover new complexities in protein export, and to improve the current *in vitro* systems such that they accurately reflect these additional features. This will undoubtedly involve avoiding reductionistic-thinking pitfalls in the short term in order to have a more correct overview in the long term.

Characterization of SecA ATPase Activity

A general requirement for ATP hydrolysis has been found in all protein translocation systems studied (for review, see Verner and Schatz, 1988). Given this fact, there was great interest when Lill *et al.* (1989) showed that SecA protein possessed an ATPase activity. Although SecA protein displayed a low level of endogenous ATPase activity, addition of SecA protein and export-competent proOmpA to urea-treated, unc$^-$, inverted plasma membrane vesicles (lacking the F1 ATPase) resulted in marked stimulation of ATPase activity. This stimulation not only required the presence of all three components in the reaction, but was also dependent on the presence of an active SecY protein in the membrane vesicles. This activity was called translocation ATPase by Wickner and associates. Initially, it was not clear whether this activity corresponded to a translocation-dependent stimulation of SecA ATPase activity or SecA-dependent activation of another membrane-bound ATPase involved in protein export. Evidence in favor of the former possibility was originally offered by showing that 8-azido-ATP derivatization of SecA protein by photocrosslinking inactivated SecA-promoted *in vitro* protein translocation and translocation ATPase activities with identical kinetics, implying that at least an ATP-binding site on SecA protein was responsible for the effects observed. Recently, it was possible to reconstitute this high level of ATPase activity using only liposomes containing SecA and export-competent proOmpA (Lill *et al.*, 1990), thereby providing more direct proof that SecA comprises the major ATPase found in translocation ATPase.

More recent enzymological work on SecA ATPase implied an interaction of both the signal peptide and mature portions of the secretory precursor protein with SecA in promoting the stimulation of the ATPase activity normally observed. It was found that addition of export-competent proOmpA was needed for stimulation of SecA ATPase activity in inverted plasma membrane vesicles, and that neither the signal peptide nor urea-denatured OmpA alone were sufficient to promote this activation. That the signal peptide was recognized in this process was implied by its competitive inhibition with proOmpA for translocation ATPase activity (Cunningham and Wickner, 1989). Precursor-independent, high-level, SecA ATPase activity was demonstrated recently in liposomes, but only below 30°C, due to thermolability of this enzyme activity. SecA ATPase activity was stabilized at higher temperatures by either proOmpA addition or addition of a mixture of signal peptide and urea-denatured, mature OmpA or maltose-binding protein, but not by addition of signal peptide or mature protein alone (Lill, *et al.*, 1990). Clearly additional work is now warranted to define the sites of these interactions on SecA protein.

Of interest in further characterization of SecA ATPase is the finding that this enzyme appears to possess three ATP-binding sites. This conclusion is based on studies which showed that SecA protein had two sites that were photocrosslinked with 8-azido-ATP and apparently one site which was not, since the completely derivatized protein continued to display the low endogenous ATPase activity. This finding is consistent with the presence of the three predicted ATP-binding sites for SecA protein shown in Fig. 1. Furthermore, these observations strengthen a possible analogy between the SecA ATPase and the *E. coli* F1 ATPase since both proteins apparently possess three ATP-binding sites (Senior and Wise, 1983; Lill *et al.*, 1989), are extremely sensitive to sodium azide inhibition (Kobayashi and Anraku, 1972; see above), and are enzymes which couple ATP hydrolysis with movement of molecules through membrane pores (Fo for F1, and possibly SecY and other integral membrane Sec components for SecA). If there is any validity to this analogy, the mechanism of sodium azide inhibition of F1 ATPase activity becomes relevant for consideration here. Sodium azide appears to inhibit an allosteric change in F1 which normally occurs upon binding an additional molecule of ATP, which results in a huge increase in the rate of ATP hydrolysis at the first bound site (a phenomenon known as multisite activation) (Noumi *et al.*, 1987). Sodium azide-inhibited F1 enzyme therefore maintains the lower level of ATP hydrolysis found when only one ATP molecule is bound per molecule of enzyme (the unisite rate). This mechanism, in fact, parallels the results of the SecA-azido-ATP derivatization studies, in that in both systems the additional ATP-binding sites would be used as allosteric effectors controlling the rate of hydrolysis at the first bound site. Clearly this prediction is testable for SecA ATPase. It will be of interest to determine whether sodium azide inactivation of SecA protein specifically inhibits its translocation ATPase activity, but not its low endogenous ATPase activity. Certainly further enzymological studies of sodium azide's effect on SecA ATPase could reveal novel properties of this enzyme.

Major challenges still remain in the characterization of SecA ATPase and discovering its role in catalyzing protein export. The creation of an *in vitro* protein translocation system in which SecA-catalyzed, ATP hydrolysis is coupled to precursor protein translocation should be a high priority. In the current system translocation ATPase activity is not properly coupled to precursor protein translocation, resulting in an artificially high stoichiometry of approximately 5000 molecules of ATP hydrolyzed per molecule of proOmpA translocated (Cunningham and Wickner, 1989). Uncoupling of this system could be due to the presence of abortively translocated precursor proteins which continue to activate SecA ATPase, thereby wasting ATP (*in vitro* translocation is usually no more than 50% efficient). Alternatively,

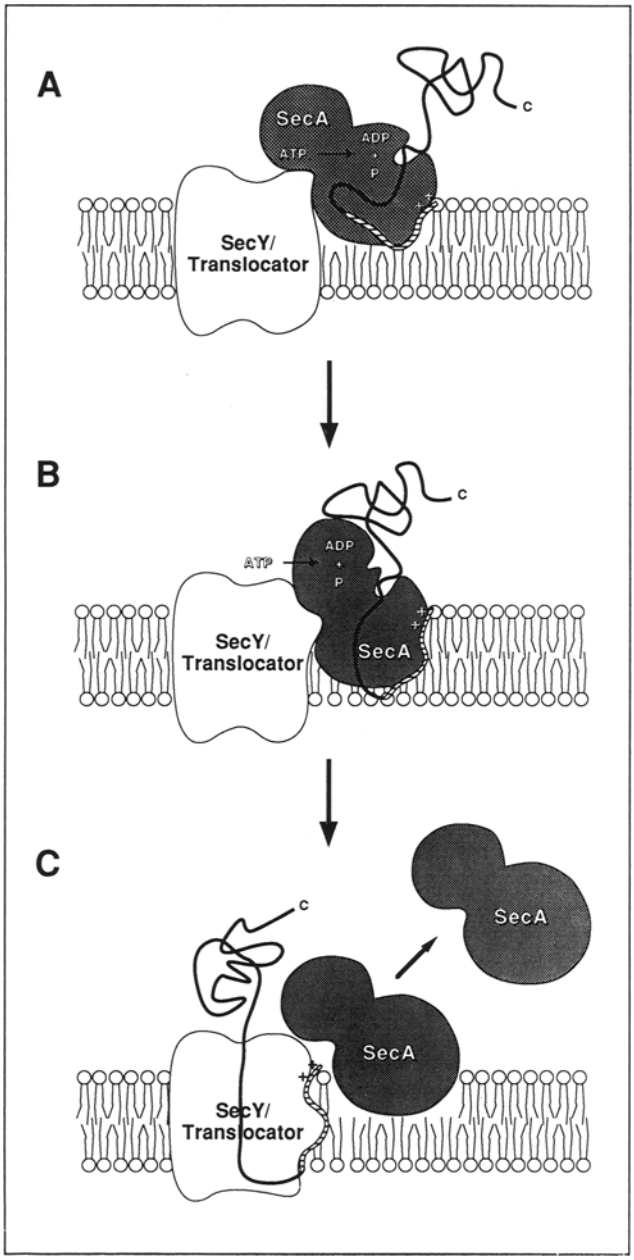

Fig. 3. Co-insertional model of SecA function. (A) SecA forms a ternary complex with the precursor protein (shown as a black ribbon with the signal peptide indicated by the stippled portion) and SecY/translocator. (B) Ternary complex formation and ATP hydrolysis promotes co-insertion of the SecA-precursor protein complex into the membrane. (C) Additional SecY/ translocator interactions and ATP hydrolysis lead to de-insertion of SecA protein from the membrane and transfer of mature segments of the precursor protein to SecY/translocator.

there may be a coupling factor which is missing or has been inactivated in the preparation of this semipurified system.

What function does ATP hydrolysis by SecA serve in promoting protein translocation across membranes? Three possible roles are considered here. First, similar to what has been postulated to occur in eukaryotic cells (for review, see Bernstein *et al.*, 1989), there may be a need to unfold secretory precursor proteins prior to or during their insertion into the membrane even if some elements of protein folding have been prevented by interaction with soluble chaperones. Second, insertion of the polypeptide chain into the membrane may often be an energetically unfavorable event, depending on the charge distribution of the polypeptide chain, and therefore require a net imput of energy. A membrane inserting/de-inserting protein which would bind the polypeptide chain, pull it into the membrane, hydrolyze ATP, and de-insert could provide the necessary driving force for this reaction (see co-insertion model below). Third, ATP hydrolysis could be used to order the macromolecular assembly of Sec machinery components and the secretory precursor protein in a committed step, similar to the role GTP hydrolysis plays in determining the specificity and order of events in the initiation of protein synthesis (Thompson, 1988).

Conclusions and Future Directions

Ingenious genetic selections allowed the isolation of *secA* mutants whose export physiology indicated that SecA was generally required for the secretion of most periplasmic and outer proteins, and for the proper insertion of integral membrane proteins containing an exported polypeptide segment. The existence of these mutants allowed the cloning and sequencing of the *secA* gene, the study of its regulation, and set the stage for a direct demonstration of the requirement for this gene product in promoting precursor protein translocation and ATP hydrolysis in *in vitro* protein translocation systems. Clearly, current work in this area promises to complete this circle of events, since biochemical elucidation of SecA protein, its ATPase activity, its interaction with membranes, the membrane-associated Sec machinery, and secretory precursor proteins should now spark a second round of genetic work aimed at the elucidation of these features of SecA protein biology.

Despite many recent and exciting developments, we are still left with much unfinished business in sorting out the roles of SecA in protein export and autogenous regulation. What does the protein do to promote membrane translocation of precursor proteins? Does it have a soluble role? What is the ATPase activity used for? What, if any, integral membrane Sec machinery components does SecA interact with and for what purpose? What elements

of precursor protein structure are recognized by SecA? Is there some sort of cycling mechanism of SecA protein within or in and out of the membrane? By what mechanism does the protein autogenously repress its own translation in coordination with the cellular export capability and for what end? Answers to these questions clearly represent a tall order.

In concluding this chapter it is only fitting to present a working model of SecA's role(s) in protein export in order to stimulate further research in this area. Our model focuses specifically on the membrane translocation step, since the targeting of secretory precursor proteins to the membrane by an SRP/SRP receptor-like mechanism and SecA's participation in this process are yet to be resolved. SecA protein, either free or in association with the precursor protein as part of an SRP-like complex, binds to the membrane in an acidic lipid-dependent fashion and moves within the plane of the membrane until it interacts with both secretory precursor and SecY proteins (and/or other integral-membrane, Sec machinery components that form the SecY translator) (Fig. 3A). Ternary complex formation triggers an ATP-dependent, conformational change in SecA, resulting in deeper insertion of portions of the precursor protein by virtue of their association with this region of SecA (Fig. 3B). The inserted SecA-precursor protein complex interacts with additional regions of the SecY translocator in this membranous environment. This results in another ATP-dependent, conformational change in SecA causing transfer of the inserted portion of the precursor protein to the SecY translocator and de-insertion of SecA sequences from the membrane (Fig. 3C). This basic cycle could be repeated for successive segments of the mature polypeptide chain of the precursor protein while SecA protein remained bound continuously to signal peptide and SecY translocator elements as anchors. Alternatively, SecA protein may promote only the initial insertion of the first mature segment of the precursor protein. In our model SecA protein promotes both precursor protein unfolding and insertion into the membrane at specific sites, generating the appropriate force by possessing both SecY translocator and precursor protein binding sites. The basic role of ATP hydrolysis is to allow SecA protein to cycle between more hydrophobic (membrane-inserted) and hydrophilic (soluble/peripheral membrane) states, which ordinarily occurs in a precursor protein and SecY translocator-dependent fashion. Testing of this model is currently underway.

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