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Minireview

Biochemical characterization of the presecretory protein translocation machinery of *Escherichia coli*

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Abstract

The protein translocation apparatus in *Escherichia coli* has been studied both genetically and biochemically. In vitro protein translocation systems involving everted membrane vesicles or reconstituted proteoliposomes have significantly contributed to biochemical clarification of the structure, mechanism and energetics of the apparatus. It is established that SecA, SecY and SecE are essential components, and play fundamental roles in the translocation reaction, and that both ATP and a proton motive force are required for the translocation. A new membrane factor, SecG, was found to participate in the formation of the apparatus, causing significant enhancement of the activity. SecD was found to play a role in the release of translocated proteins from the outer surface of the cytoplasmic membrane.

Key words: Protein translocation; Sec apparatus; *Escherichia coli*; Reconstitution; Everted membrane vesicle

1. Introduction

Proteins destined to be localized in the periplasm or the outer membrane of *Escherichia coli* are synthesized as precursors comprising the mature proteins with a signal sequence at their N-termini. Translocation of these precursors across the cytoplasmic membrane is catalyzed by a machinery comprising Sec proteins. Involvement of Sec proteins in the translocation reaction has been suggested by genetic studies [1,2]. Biochemical studies were then carried out to demonstrate the direct involvement of these factors and to analyse their roles, using in vitro protein translocation systems involving everted membrane vesicles [3,4] or reconstituted proteoliposomes [5,6]. It is now certain that SecA, SecY and SecE are essential for and play central roles in the translocation of general presecretory proteins. Several reviews have been recently published on this subject [1–4,7–9]. This short review, therefore, focuses only on recent progress in the biochemistry of the Sec machinery.

2. Functions of Sec proteins

All the Sec proteins, SecA, SecB, SecD, SecE, SecF, SecG and SecY, have been overproduced, purified and subjected to biochemical analyses. SecA and SecB have been purified from the cytosol, whereas the others are integral membrane proteins and have been isolated from solubilized membrane extracts. SecG, formerly termed p12, is a new factor that was discovered in reconstitution studies [10] and proved to be involved in protein translocation by in vivo genetic studies (manuscript submitted).

SecA, a 102-kDa protein, is found both in the cytosol and on membranes, and plays a key role in the initiation of protein translocation. SecA interacts with a presecretory protein, ATP, acidic phospholipids and, presumably, SecY and/or SecE [11–14]. Interaction with these factors causes a structural change of SecA [15], which is likely to cause insertion of the presecretory protein together with SecA into the phospholipid bilayer [16,17]. This is discussed in more detail in a later section.

Chemical cross-linking studies demonstrated that ATP and the presecretory protein bind to the N-terminal region of SecA [11,12]. The N-terminal positive charge of the signal peptide is important for the interaction with SecA [11]. Recent site-directed mutagenesis suggested that SecA possesses two ATP binding sites; an N-terminal high-affinity binding site corresponding to the one identified in a cross-linking study, and a low affinity binding one located in the middle of the SecA molecule [18]. The precursor binding site identified previously is located between these two ATP binding sites. Both sites are suggested to be essential for SecA-dependent protein translocation. Although the roles of the individual ATP binding sites are not clear, it is likely that one ATP binding site is directly involved in the translocation-coupled ATP hydrolysis and the other one functions as a regulatory site.

Among the 7 Sec proteins, only SecA exhibits ATP hydrolysing activity. Phosphorylation has not been reported with any Sec proteins. SecA exhibits both endogenous and presecretory protein-dependent ATPase

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Abbreviations: Δp, proton motive force; MBP, maltose binding protein; OmpA, outer membrane protein A; ER, endoplasmic reticulum; SRP, signal recognition particle; SR, SRP receptor.

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activities, the latter of which requires everted membrane vesicles [19]. The role of ATP hydrolysis by SecA in protein translocation is discussed in a later section.

SecB maintains a certain number of presecretory proteins in a translocation-competent state [20], whereas it is not essential for cell viability. SecB does not directly comprise the translocation machinery and, therefore, is not discussed in detail here.

Early biochemical studies involving membrane vesicles suggested the importance of SecY for protein translocation [21]. Direct involvement of SecY and SecE in the translocation reaction has been demonstrated by reconstitution studies. Protein translocation activity, which is dependent on SecA and ATP, was reconstituted into proteoliposomes from the purified SecY/SecE complex [5] or independently purified SecY and SecE [6]. The essentiality of SecY and SecE as well as SecA has thus been established.

SecY has a molecular mass of 49 kDa and is suggested to span the cytoplasmic membrane 10 times [22]. Since this structure resembles those of solute transport membrane proteins such as lactose permease [23], SecY is thought to form a channel through which presecretory proteins are translocated across the membrane. This has not yet been demonstrated, however. SecE, a 14 kDa protein, is thought to span the membrane three times [24]. Both genetic [25] and biochemical [26] studies revealed that the SecE function exists in the third transmembrane segment and preceding cytoplasmic region. Interestingly, a SecE homologue in *Bacillus subtilis* retains only one transmembrane segment [27]. A certain number of amino acid residues in the preceding cytoplasmic region are conserved in the two organisms.

SecY and SecE, most likely, function as a complex. Interaction between the two Sec proteins has been indicated by the following observations; SecY and SecE can be isolated as a complex [5], and overproduction of SecY is possible when SecE is overproduced at the same time [28]. The isolated SecY/SecE complex, whether chromatographically purified or immunochemically precipitated with an anti-SecY antibody, contained another protein, termed band 1 [5,29]. The role of band 1 in the protein translocation was not clear until recently since the translocation machinery can be reconstituted with highly purified SecY, SecE and SecA, without other proteins [6]. The activity of proteoliposomes reconstituted with the three Sec proteins was, however, considerably lower than that of membrane vesicles. SecG, formerly termed p12, was found as a new component which stimulates the reconstituted activity [10]. The nucleotide sequence of the gene encoding SecG has been determined [10]. Disruption of this gene impaired the protein translocation in vivo (manuscript submitted), indicating that SecG is directly involved in protein translocation in *E. coli* (Fig. 1). Immunochemical studies involving an antibody raised against a partial sequence of SecG revealed recently that SecG is identical to band 1 (unpublished observation).

The protein translocation machinery, the Sec61p complex, in the membrane of the mammalian ER was reported to comprise three protein subunits [30]. The sequences of the α and γ subunits exhibit some similarity to those of SecY and SecE, respectively [31,32]. These observations indicate the evolutionary conservation of components of the protein translocation machinery from prokaryotes to mammals.

Both SecD and SecF have been suggested by genetic studies to be involved in a late step of protein translocation [33,34]. The direct participation of SecD in this step was demonstrated with spheroplasts by means of an immunochemical technique [35]. Treatment of spheroplasts with an antibody raised against SecD inhibited the secretion of several proteins, such as MBP and OmpA, into the medium [35]. Both the precursor and mature forms of secretory proteins were accumulated in the antibody-treated spheroplasts. The mature MBP that accumulated in the spheroplasts was shown to be sensitive to trypsin, whereas that secreted into the medium was resistant, suggesting that the trypsin-sensitive mature MBP existed in an unfolded conformation on the surface of the spheroplasts. From these results, SecD was concluded to play a role in the release of translocated proteins from the outer surface of the cytoplasmic membrane (Fig. 1). On the other hand, the exact function of SecF is not clear. This protein exists in membranes at an about 10-fold lower level than other membrane Sec proteins [9]. SecF may have a regulatory function.

3. Energy requirement

Protein translocation in *E. coli* requires two energy sources, ATP and a proton motive force (Δp). ATP is essential for the initiation of protein translocation, whereas translocation in vitro takes place, though at a significantly reduced rate, in the absence of Δp. On the other hand, after a certain stage of protein translocation, Δp alone can drive the translocation to completion [36,37]. These observations suggest that the initiation of protein translocation caused by the insertion of a presecretory protein into the lipid bilayer is mechanistically different from the translocation of an ongoing peptide (Fig. 1).

SecA has been shown to penetrate the lipid bilayer upon interaction with acidic phospholipids [16,17]. The binding of ATP to the SecA-presecretory protein complex is suggested to cause the insertion of the presecretory protein into the lipid bilayer [37]. Subsequent hydrolysis of ATP is supposed to cause the transfer of the presecretory protein from SecA to membrane Sec proteins (SecY and SecE), and then the release of SecA from the membrane. Based on these observations, transloca-

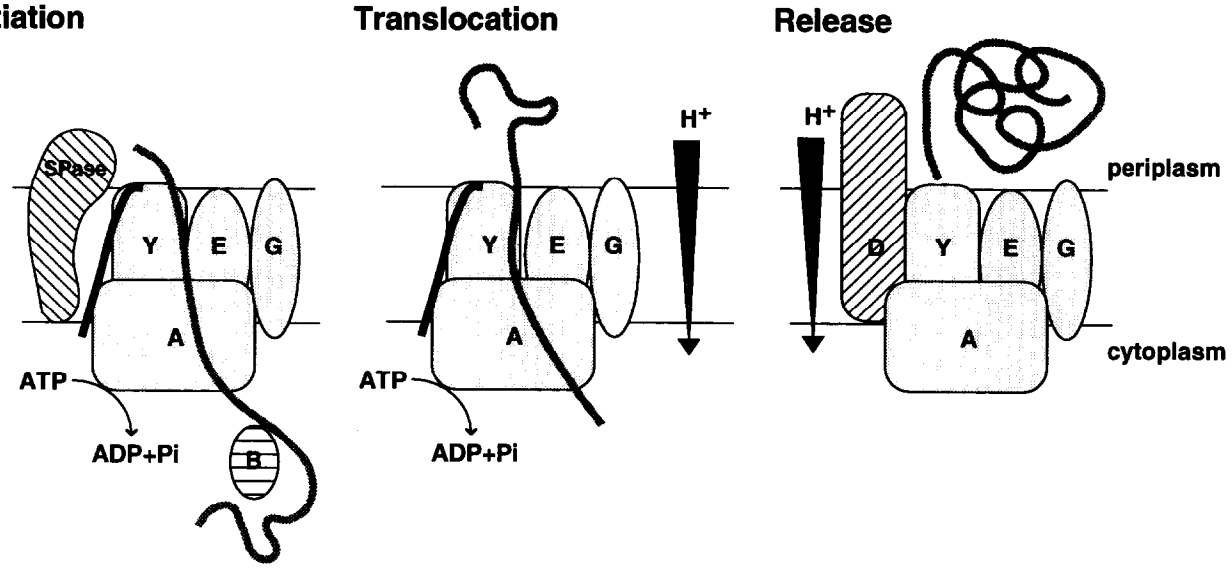

Fig. 1. Schematic model of protein translocation catalyzed by the Sec machinery. Protein translocation is divided into three steps. Sec proteins participating in each step are denoted by capital letters. SecA and SecB presumably exist as a homodimer and homotetramer, respectively. Other stoichiometries are unknown. The energy proposed to be involved in each step is also indicated. ATP-dependent insertion of a presecretory protein into the lipid bilayer is supposed to make the cleavage site of the presecretory protein accessible to signal peptidase, which has its catalytic domain in the periplasm. SPase, signal peptidase; H^+➡, Δp. For details, see the text.

tion of an entire protein molecule has been proposed to be performed through successive cycles of SecA-precursor binding, ATP binding and hydrolysis, and precursor-transfer to membrane components of the machinery [37]. Each cycle is assumed to cause the translocation of a short peptide segment. Although further critical studies are required to confirm this, this model explains the early event of protein translocation.

Δp, which is composed of a membrane potential ($\Delta\Psi$) and ΔpH, causes significant stimulation of the translocation of all presecretory proteins so far examined, including one which has no charged amino acid residues in its mature region [38]. This excludes the electrophoretic movement of the negatively charged portions of secretory proteins toward the periplasm as the main reason for the stimulation by $\Delta\Psi$ (positive on the periplasmic side). Furthermore, deprotonation of basic amino acid residues before translocation is not the main reason for the stimulation of the translocation by ΔpH (acidic on the periplasmic side). The machinery itself seems, therefore, to be in an efficient (or energized) state in the presence of Δp. The following may represent properties of the machinery in this energized state; the translocation machinery exhibits higher affinity for ATP in the presence of Δp than in its absence [39], and proOmpA, having a disufide-bonded loop in its C-terminal region, can only be translocated in the presence of Δp [40]. If energization of protein translocation takes place in a similar manner to that of solute transport systems, protonflux should be coupled to protein translocation. Although observations suggesting proton transfer is coupled to the translocation have been reported [41,42], it is still too early to draw a definite conclusion from these observations. Requirement of Δp for the release of a translocated protein from the outer surface of the cytoplasmic membrane has also been suggested [43,44].

4. Problems remaining to be clarified

SRP and its receptor, SR, are involved in protein translocation across the ER membrane of eukaryotic cells. Ffh, FtsY and 4.5 S RNA found in *E. coli* structurally resemble the 54 kDa subunit of SRP, the α subunit of SR, and 7 S RNA of SRP, respectively. It has been reported that Ffh is important for protein translocation in vivo [45]. Furthermore, the Ffh/4.5 S RNA complex, having an SRP-like function, was found to bind to FtsY in a GTP-dependent manner [46]. This interaction caused the stimulation of GTP hydrolysis, which signal peptides inhibited [46]. These properties resemble those of the eukaryotic system, suggesting that the *E. coli* system functions in protein targeting in a similar manner to that of eukaryotic counterparts. On the other hand, SecA and SecB have been shown to function in precursor recognition and targeting [47]. The extent of the contribution of the *E. coli* SRP homologue to protein translocation and its relation to the Sec apparatus remain to be clarified.

By means of an electrophysiological technique, a channel, which opens upon the addition of a signal peptide, was detected in *E. coli* membranes [48]. It is not

known, however, whether or not any Sec proteins are involved in the channel activity. The opening of such a channel should immediately result in the complete collapse of Δp. Although the observation of this signal peptide-dependent channel is intriguing, further studies are required to draw a definite conclusion.

Considerable amounts of information have accumulated as to the structure and mechanism of protein translocation across the *E. coli* membrane. In contrast, little is known about the mechanism whereby translocated proteins are retained in the periplasm or transported further into the outer membrane. Factors involved in this sorting step need to be characterized.

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