View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

FEBS Letters 581 (2007) 1859–1864

# Arginine 357 of SecY is needed for SecA-dependent initiation of preprotein translocation

Jeanine de Keyzer[1], Anouk Regeling, Arnold J.M. Driessen*

*Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands*

Received 23 February 2007; revised 22 March 2007; accepted 27 March 2007

Available online 9 April 2007

Edited by Felix Wieland

**Abstract** **The *Escherichia coli* SecYEG complex forms a transmembrane channel for both protein export and membrane protein insertion. Secretory proteins and large periplasmic domains of membrane proteins require for translocation in addition the SecA ATPase. The conserved arginine 357 of SecY is essential for a yet unidentified step in the SecA catalytic cycle. To further dissect its role, we have analysed the requirement for R357 in membrane protein insertion. Although R357 substitutions abolish post-translational translocation, they allow the translocation of periplasmic domains targeted co-translationally by an N-terminal transmembrane segment. We propose that R357 is essential for the initiation of SecA-dependent translocation only.**
**© 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.**

*Keywords:* SecA; SecY; Membrane insertion; Protein translocation

## 1. Introduction

Translocation of secretory proteins across and insertion of membrane proteins into the cytoplasmic membrane of *Escherichia coli* occurs via the SecYEG protein conducting channel. Secretory proteins are targeted post-translationally to the SecYEG-bound motor protein SecA that initiates and drives translocation in an ATP dependent manner [1,2]. Most membrane proteins are targeted to SecYEG by the co-translational SRP-pathway (reviewed in [3]). Insertion of hydrophobic transmembrane segments (TMSs) and translocation of small periplasmic loops is coupled to chain elongation at the ribosome [4,5], while large extracellular domains require the assistance of SecA [6,7].

The interaction between the SecYEG channel and its cytosolic binding partners is critical for efficient protein translocation and membrane insertion. SecA and the ribosome not only drive translocation/insertion, but might also induces the conformational changes in SecYEG that are required for opening of the channel [8]. Genetic [9,10] and cross-linking [11,12] studies suggest an important role for the cytoplasmic domains of SecY in the interaction with SecA. A conserved arginine (R357) at the tip of the 5th cytoplasmic (C5) loop of SecY seems to be of special importance for the functional SecA–SecYEG interaction. Substitution by any other amino acid severely blocks protein translocation [9]. This defect is accompanied by a decreased formation of a translocation-specific protease protected fragment of SecA [12], an interference with SecYEG oligomerization and movement of the plug domain of the SecYEG channel [13]. Since hyperactive SecA mutants can relieve the detrimental effect on translocation, it was suggested that R357 is important for efficient interaction with SecA [9]. However, in vivo cross-linking of SecA to cytosolic SecY domains is not affected by histidine substitution of R357 [11] and R357 substitutions do not significantly reduce the binding of SecA to SecYEG [12], suggesting that R357 mutations interfere with a step in the translocation after the initial binding of SecA to SecYEG [12].

The effect of R357 substitutions has mainly been addressed in studies on the translocation of secretory proteins. Large periplasmic domains of membrane proteins also require SecA for translocation but it is not clear if they are affected in a similar manner. Here, we report on the effect of R357 substitutions on the SecA-dependent insertion of the type II integral membrane protein FtsQ. The data demonstrate that once insertion has been initiated, SecA-dependent translocation of the periplasmic domain of FtsQ occurs normally with R357 mutants of SecY. It is concluded that this residue of SecY is essential only for the efficient SecA-dependent initiation of post-translational translocation.

## 2. Materials and methods

### 2.1. Materials

Inner membrane vesicles (IMVs) containing wild-type or overproduced levels of SecYEG, SecY (R357E)EG, SecY (R357C)EG and SecY(R357H)EG were isolated from *E. coli* SF100 transformed with pET610 [14], pEK48 [12], pNN260 [12], and pEK49, respectively. SecA, SecB and proOmpA(C290S) were purified as described [15].

### 2.2. Plasmids

proOmpA was placed behind a T7 promoter by transferring the *Hin*dIII × *Eco*RI fragment of plasmid pET24 (J.P. van der Wolk, unpublished) into pBS2KS (Stratagene, La Jolla, USA) (pET2337). From this plasmid, DNA fragments with a silent AgeI site around Y8 of

*Corresponding author. Fax: +31 50 3632154.
*E-mail address:* a.j.m.driessen@rug.nl (A.J.M. Driessen).

[1]Present address: Faculty of Life Sciences, Michael Smith Building, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom.

*Abbreviations:* IMVs, inner membrane vesicles; SRP, signal recognition particle; TMS, transmembrane segment; PK, protease K

0014-5793/$32.00 © 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2007.03.081

mature OmpA were generated by PCR mutagenesis and used to replace the corresponding fragments in pBSKFtsQ [16]. The resulting plasmids were used for the expression of FtsQ-proOmpA chimeras in which the N-terminal 62 amino acids of FtsQ were replaced by the N-terminal 29 amino acids of proOmpA (pEK705) and vice versa (pEK706). *MtlA* was PCR amplified from plasmid pMamtlaPr6H and cloned in pET20b (Novagen), yielding pET20MtlA.

### 2.3. In vitro transcription–translation insertion and translocation

In vitro translocation of fluorescein labeled proOmpA(C290S) [17] and transcription–translation-insertion of FtsQ, FtsQ chimers and MtlA [16] were performed as described except that transcription and translation was coupled.

## 3. Results

### 3.1. The SecY(R357E) mutation affects SecA-mediated protein translocation and membrane protein insertion differently

Saturation mutagenesis indicates that any substitution of R357 of SecY severely interferes with protein translocation [9], with the substitution to glutamate being in particular destructive [9,12,13]. Therefore, we focused on the SecY(R357E) mutant to study the role of R357 in SecA-dependent membrane protein insertion. IMVs containing overexpressed SecY(R357E)EG were isolated and their protein translocation and membrane insertion activities were studied. In agreement with earlier reports, post-translational translocation of purified (fluorescently labelled) proOmpA into SecY(R357E)EG$^+$ IMVs barely exceeded the translocation into IMVs containing endogenous (non-overexpression) levels of SecYEG (Fig. 1) [12]. However, the single spanning type II membrane protein FtsQ, that requires SecA for translocation of its periplasmic domain [16], inserted at almost similar levels in SecY(R357E)EG$^+$ and SecYEG$^+$IMVs (Fig. 1).

Translocation is assayed with chemical amounts of urea-denatured proOmpA that are added to a minimal system consisting of IMVs, SecA and SecB and ATP. In contrast, membrane insertion of FtsQ is assayed in co-translationally wherein its synthesis in a cell lysate is directly coupled to its insertion into IMVs. To exclude that the efficient insertion of FtsQ in SecY(R357E)EG$^+$ IMVs was caused by the experimental set-up, translocation of proOmpA was assayed under similar conditions as FtsQ. Even when the proOmpA was synthesized in vitro in the presence of IMVs, proOmpA failed to translocate efficiently into the SecY(R357E)EG$^+$ IMVs (Fig. 1). These data indicate that the R357E mutation of SecY affects SecA dependent protein translocation and membrane protein insertion differently.

### 3.2. The SecY R357E mutant allows SecA-dependent translocation of co-translationally targeted substrates

ProOmpA and FtsQ both require SecA for translocation but are directed to the translocase by different pathways due to their N-terminal targeting signal. Whereas proOmpA is targeted post-translationally, FtsQ is targeted co-translationally [18]. To analyse whether the efficient insertion of FtsQ by SecY(R357E) also requires co-translational targeting, chimeras were constructed in which the N-terminal 63 residues (transmembrane segment) of FtsQ and the N-terminal 32 residues (signal sequence) of proOmpA were exchanged. This resulted in proteins in which the periplasmic domain of FtsQ was preceded by the signal sequence of proOmpA (proFtsQ) or in which the mature domain of OmpA was preceded by the transmembrane segment of FtsQ (TMSOmpA). Translocation/insertion of these chimers was tested by their protease sensitivity after in vitro synthesis in the presence of SecYEG$^+$ IMVs (Fig. 2A, left panel, co-translational). Translocation of proFtsQ resulted in the protection of the full-length protein and partial processing of the signal sequence. Membrane insertion of TMSOmpA resulted in a truncation consistent with degradation of the short cytoplasmic FtsQ-domain and protection of the TMS and OmpA domain. Both translocated proFtsQ and inserted TMSOmpA were completely degraded when membranes where solubilised with the detergent Triton X-100 (see Fig. 2C). This demonstrates that proFtsQ and TMSOmpA are correctly translocated and/or inserted into the IMVs. In contrast to the insertion of the SecA independent membrane protein MtlA, translocation of both chimeras was inhibited by azide and thus dependent on SecA (Fig. 2B). Both

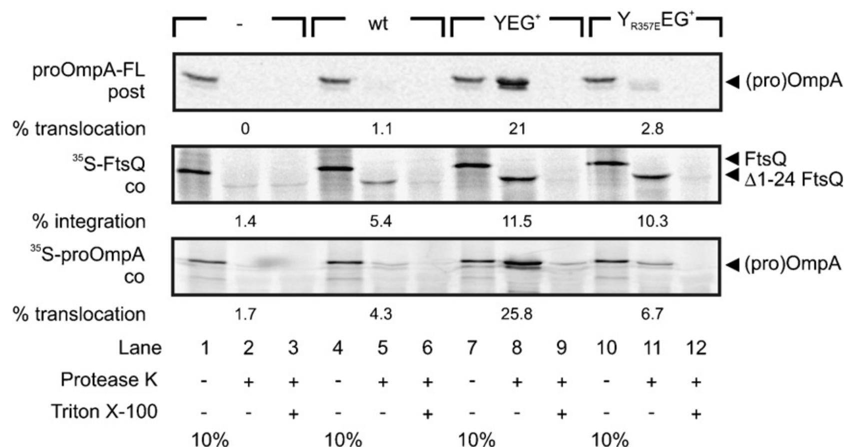


Fig. 1. SecY(R357E) supports the efficient insertion of the SecA dependent membrane protein FtsQ. Translocation of fluorescein or $^{35}$S-labeled proOmpA and membrane insertion of $^{35}$S-labeled FtsQ assayed post-translationally (post) or co-translationally (co). Assays were performed in the absence (–) or in the presence of IMVs containing endogenous levels of SecYEG (wt) or overexpressed SecYEG (YEG$^+$) or SecY(R357E)EG ($Y_{R357E}EG^+$) in a wild-type background. Correct FtsQ membrane insertion protects the TMS and periplasmic domain, but not the short cytoplasmic domain, from protease K digestion [16]. The low level of insertion or translocation in the absence of IMVs is caused by IMVs that are present in the S135 lysate. For non-protease K treated samples 10% of the total translation is shown.

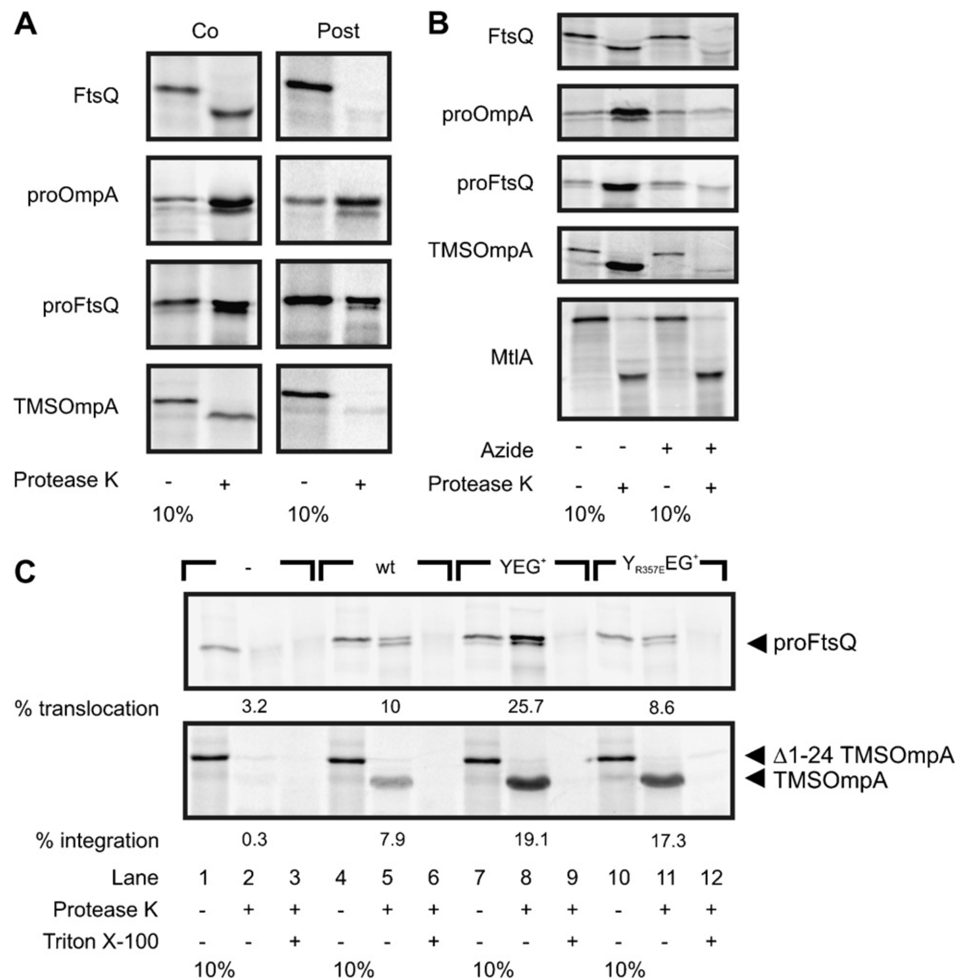


Fig. 2. Efficient SecA-dependent translocation by SecY(R357E) requires co-translational targeting. (A) Co-(left panels) and post-(right panels) translational translocation/insertion of FtsQ, proOmpA, proFtsQ and TMSOmpA into SecYEG+ IMVs. For post-translational translocation, proteins were synthesized in vitro in the absence of IMVs, denatured by the addition 6 M urea and diluted into a standard protein translocation reaction mixture. (B) Translocation/insertion of FtsQ, proOmpA, proFtsQ, TMSOmpA and MtlA in the presence or absence of 20 mM sodium azide. (C) Translocation/insertion of FtsQ, proOmpA, proFtsQ or TMSOmpA.

chimeras required the proton motive force for translocation (data not shown).

To determine whether the proteins were translocated co-or post-translationally, they were first synthesized in vitro in the absence of IMVs, denatured with urea and added to a translocation reaction with IMVs, SecA, SecB and ATP. Under these post-translational conditions, only the signal sequence containing proFtsQ and proOmpA proteins were translocated efficiently (Fig. 2A, right panel), whereas the co-translational substrates FtsQ and TMSOmpA were not translocated. Next, the ability of the chimers to be translocated by SecY(R357E)EG was tested. Like with FtsQ, TMSOmpA was inserted with an almost similar efficiency into SecY(R357E)EG+ and SecYEG+ IMVs (Fig. 2C). In contrast, the translocation of proFtsQ was abolished to background insertion levels that are due to the endogenous SecYEG (Fig. 2C). These data demonstrate that the presence of the hydrophobic transmembrane segment in a protein substrate circumvents the translocation defect of SecY(R357E)EG, which suggests that this mutation interferes with translocation at the level of targeting and translocation initiation.

### 3.3. The SecY R357C mutant exhibits a general translocation and insertion defect

Since genetic studies indicate that any substitution of R357 is detrimental for protein translocation [9], the effect of two other substitutions of R357 was analysed. Like the R357E mutation, substitution to a cysteine or histidine severely affected the translocation of proOmpA (Fig. 3A and [12]). In contrast to earlier observations [9], the histidine mutant appears even more defective in protein translocation than the glutamate mutant. Despite its severe effect on protein translocation (Fig. 3A), the R357H mutant still supported a substantial level of FtsQ insertion (Fig. 3B) similar to the glutamate mutant (Fig. 3B). In contrast, IMVs containing the R357C mutant were slightly more active in protein translocation than the other mutants (Fig. 3A, [12]). However, SecY(R357C) IMVs did not show any insertion of FtsQ above the level mediated by the endogenous SecYEG complex (Fig. 3B). To test if this severe insertion defect was due to an aberrant interaction with SecA, insertion of the SecA-independent membrane protein MtlA was assayed. MtlA is a polytopic membrane protein of which a 30 kDa fragment becomes protected for proteases

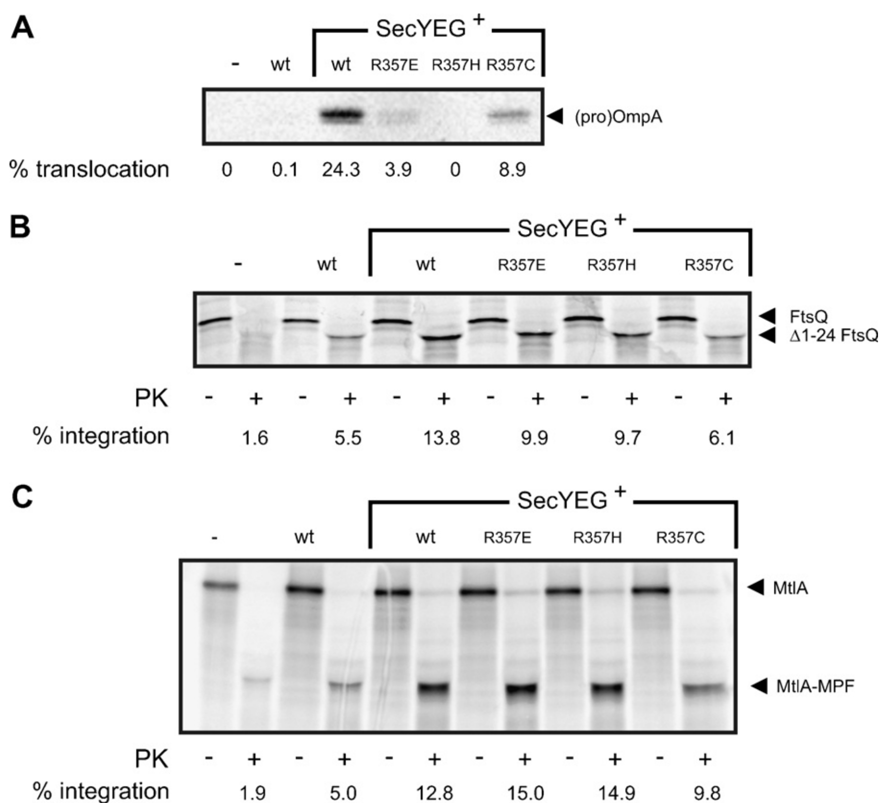


Fig. 3. The SecY(R357C) mutant is defective in membrane protein insertion. Post-translational translocation of proOmpA (A), co-translational insertion of FtsQ (B) and co-translational insertion of MtlA (C) by SecY-R357 mutants. MtlA insertion is assayed similar as FtsQ. PK: protease K. For non-protease K treated samples 10% of the total translation is shown.

upon correct insertion into the membrane [19]. While MtlA insertion was not affected by substitution of R357 to arginine or histidine, it was reduced upon substitution to cysteine (Fig. 3C). This demonstrates that the R357C mutation impairs SecY in a manner unrelated to the catalytic activity of SecA.

## 4. Discussion

Several studies demonstrate that the arginine at position 357 in the 5th cytosolic loop of SecY is essential for a functional SecA reaction cycle [9,12,13]. Since the physical interaction between SecA and the SecYEG complex is unaffected in mutants of this residue [12], it has remained unclear at what step of the translocation reaction R357 is required. The observation that substitution of R357 to glutamate or histidine does not affect the correct localization of the SecA-dependent membrane protein FtsQ allowed us to discriminate between a role of this residue at an initial and later stage of translocation. As will be discussed below, we propose that the arginine at position 357 is required only at an early SecA-dependent step in the translocation reaction.

Although the exact events that underlie the initiation of translocation and insertion have not yet been elucidated, recent insights in the structure of the SecYEG complex and its cytosolic binding partners suggest a possible mechanism of channel opening. Depending on the targeting pathway, the first cytosolic factor that associates with SecYEG and initiates insertion or translocation is either the ribosome (co-translational) or SecA (post-translational). A recent model, based on the analysis of structures of the ribosome and the SecYEG complex, proposes that the ribosome plays an active role in regulation of the channel opening. The ribosome may sense the signal sequence or transmembrane segment(s) of nascent polypeptides at an early stage, whereupon it could undergo conformational changes that are propagated to the SecYEG complex causing the opening of the protein conducting channel [8,20]. In analogy, ligand-induced conformational changes of SecA may result in channel opening in a manner similar to that proposed for the ribosome [8]. The C5-loop of SecY may play an important role in the propagation of such translocation-initiation-signals from SecA (and the ribosome). However, once the channel is in a translocation active state, residues that are essential for this initial SecA–SecY communication may be less critical for function.

Previous studies have shown that the hydrophobic transmembrane segment of nascent FtsQ is recognized by signal recognition particle (SRP) whereupon the protein is directed into the co-translational targeting pathway [18]. Cross-linking studies indicate that SecA is recruited for the translocation of the periplasmic domain of FtsQ after insertion of the TMS [21]. Our data demonstrate that the R357 glutamate and histidine mutations in SecY do not interfere with this process, possibly because the SecYEG-channel is already primed for translocation by the ribosome. The observation that the R357H and R357E mutants only affect substrates of which the translocation is initiated by SecA suggests a functional role of R357 at the initial stages of post-translational protein translocation.

Structural [22] and biochemical [23] data indicate that the C5-loop is also involved in ribosome binding. The ribosome

binding affinity of Sec61p, the SecY homologue of *Saccharomyces cerevisiae*, is reduced by mutation of the residue corresponding to *E. coli* R357 (Sec61p R406) [24]. This may explain why FtsQ and MtlA fail to insert efficiently in the R357C mutant, whereas this mutant still allowed a low level of proOmpA translocation (this study, [12]). The R357H mutation in the chromosomal *secY* gene (*secY39cs*) has been reported to result in membrane protein insertion defects at low temperatures (25–30 °C) [25,26]. At physiological temperature, however, the R357H and R357E mutations have little impact on the in vitro insertion of FtsQ and the SecA-independent membrane protein MtlA. A recent study shows that *secY39cs* also effectively inserts LacY and fusions with SecY and MalF [27]. Thus, the role of R357 in membrane protein insertion appears less critical as in protein translocation. How can this be understood? The binding affinity of SecY (and of SecY mutants) for non-translating ribosomes might differ from the affinity for nascent chain containing ribosomes. For instance, translating ribosomes are still able to interact with SecYEG even when the C-4 and C-5 loops have been digested by protease, whereas these SecYEG-complexes were unable to interact with non-translating ribosomes [23]. Alternatively, disruption of a single contact between SecY and the ribosome may not be sufficient to inactivate ribosome mediated co-translational translocation as the ribosome–SecYEG interaction involves multiple sites of contact [22,28–31]. SecA also likely interacts with multiple SecY regions as it can be crosslinked to nearly all cytosolic domains of SecY [27], but apparently, for functional translocation at the initiation stage, R357 appears highly critical.

Instead of being strictly required for SecA-dependent initiation of translocation, one could argue that R357 is involved directly in signal sequence recognition while it would not be needed for the binding of transmembrane segments. We consider this explanation less likely as the basic mechanism of channel opening is generally assumed to be similar for membrane proteins and secretory proteins. Neither does it explain why R357 is so non-promiscuous that even the conservative substitution into a lysine severely impairs protein translocation [9].

Taken together, the present analysis of the effect of R357 mutations on the insertion of a SecA dependent membrane protein has allowed us to dissect its role in the initial and later stages of translocation. Once translocation or insertion has been initiated, R357 is no longer critical for SecA-dependent translocation. This amino acid residue may be strictly involved in the initial SecA-dependent channel opening or formation mechanism. Future studies should address the molecular mechanism by which a single amino acid substitution at SecY can deregulate the initiation of protein translocation.

*Acknowledgements:* We thank Francois du Plessis, Nico Nouwen and Stef Kol for stimulating discussions and technical assistance. Elisa Vervoort and Bert Poolman are thanked for their kind gift of pMamtlaPr6H. This work was supported by European Community Grant LSHG-CT-2004-504601 (E-Mep).

## References

[1] Cabelli, R.J., Chen, L., Tai, P.C. and Oliver, D.B. (1988) SecA protein is required for secretory protein translocation into *E. coli* membrane vesicles. Cell 55, 683–692.

[2] Schiebel, E., Driessen, A.J.M., Hartl, F.U. and Wickner, W. (1991) ΔμH+ and ATP function at different steps of the catalytic cycle of preprotein translocase. Cell 64, 927–939.

[3] Herskovits, A.A., Bochkareva, E.S. and Bibi, E. (2000) New prospects in studying the bacterial signal recognition particle pathway. Mol. Microbiol. 38, 927–939.

[4] Scotti, P.A., Valent, Q.A., Manting, E.H., Urbanus, M.L., Driessen, A.J.M., Oudega, B. and Luirink, J. (1999) SecA is not required for signal recognition particle-mediated targeting and initial membrane insertion of a nascent inner membrane protein. J. Biol. Chem. 274, 29883–29888.

[5] Neumann-Haefelin, C., Schafer, U., Muller, M. and Koch, H.G. (2000) SRP-dependent co-translational targeting and SecA-dependent translocation analyzed as individual steps in the export of a bacterial protein. EMBO J. 19, 6419–6426.

[6] Lee, J.I., Kuhn, A. and Dalbey, R.E. (1992) Distinct domains of an oligotopic membrane protein are Sec-dependent and Sec-independent for membrane insertion. J. Biol. Chem. 267, 938–943.

[7] Andersson, H. and von Heijne, G. (1993) Sec dependent and Sec independent assembly of E. coli inner membrane proteins: the topological rules depend on chain length. EMBO J. 12, 683–691.

[8] Mitra, K., Frank, J. and Driessen, A.J.M. (2006) Co- and post-translational translocation through the protein-conducting channel: analogous mechanisms at work? Nat. Struct. Mol. Biol. 13, 957–964.

[9] Mori, H. and Ito, K. (2001) An essential amino acid residue in the protein translocation channel revealed by targeted random mutagenesis of SecY. Proc. Natl. Acad. Sci. USA 98, 5128–5133.

[10] Matsumoto, G., Nakatogawa, H., Mori, H. and Ito, K. (2000) Genetic dissection of SecA: suppressor mutations against the secY205 translocase defect. Genes Cells 5, 991–999.

[11] Mori, H. and Ito, K. (2006) Different modes of SecY–SecA interactions revealed by site-directed in vivo photo-cross-linking. Proc. Natl. Acad. Sci. USA 103, 16159–16164.

[12] van der Sluis, E.O., Nouwen, N., Koch, J., de Keyzer, J., van der Does, C., Tampé, R. and Driessen, A.J.M. (2006) Identification of two interaction sites in SecY that are important for the functional interaction with SecA. J. Mol. Biol. 361, 839–849.

[13] Tam, P.C., Maillard, A.P., Chan, K.K. and Duong, F. (2005) Investigating the SecY plug movement at the SecYEG translocation channel. EMBO J. 24, 3380–3388.

[14] Kaufmann, A., Manting, E.H., Veenendaal, A.K.J., Driessen, A.J.M. and van der Does, C. (1999) Cysteine-directed cross-linking demonstrates that helix 3 of SecE is close to helix 2 of SecY and helix 3 of a neighboring SecE. Biochemistry 38, 9115–9125.

[15] van der Does, C., de Keyzer, J., van der Laan, M. and Driessen, A.J.M. (2003) Reconstitution of purified bacterial preprotein translocase in liposomes. Methods Enzymol. 372, 86–98.

[16] van der Laan, M., Nouwen, N. and Driessen, A.J.M. (2004) SecYEG proteoliposomes catalyze the $\Delta\psi$-dependent membrane insertion of FtsQ. J. Biol. Chem. 279, 1659–1664.

[17] de Keyzer, J., van der Does, C. and Driessen, A.J.M. (2002) Kinetic analysis of the translocation of fluorescent precursor proteins into *Escherichia coli* membrane vesicles. J. Biol. Chem. 277, 46059–46065.

[18] Valent, Q.A., de Gier, J.W., von, H.G., Kendall, D.A., ten Hagen-Jongman, C.M., Oudega, B. and Luirink, J. (1997) Nascent membrane and presecretory proteins synthesized in *Escherichia coli* associate with signal recognition particle and trigger factor. Mol. Microbiol. 25, 53–64.

[19] Werner, P.K., Saier Jr., M.H. and Muller, M. (1992) Membrane insertion of the mannitol permease of *Escherichia coli* occurs under conditions of impaired SecA function. J. Biol. Chem. 267, 24523–24532.

[20] Mitra, K. and Frank, J. (2006) A model for co-translational translocation: ribosome-regulated nascent polypeptide translocation at the protein-conducting channel. FEBS Lett. 580, 3353–3360.

[21] Urbanus, M.L., Scotti, P.A., Froderberg, L., Saaf, A., de Gier, J.W., Brunner, J., Samuelson, J.C., Dalbey, R.E., Oudega, B. and Luirink, J. (2001) Sec-dependent membrane protein insertion: sequential interaction of nascent FtsQ with SecY and YidC. EMBO Rep. 2, 524–529.

[22] Mitra, K., Schaffitzel, C., Shaikh, T., Tama, F., Jenni, S., Brooks III, C.L., Ban, N. and Frank, J. (2005) Structure of the *E. coli* protein-conducting channel bound to a translating ribosome. Nature 438, 318–324.

[23] Raden, D., Song, W. and Gilmore, R. (2000) Role of the cytoplasmic segments of Sec61alpha in the ribosome-binding and translocation-promoting activities of the Sec61 complex. J. Cell Biol. 150, 53–64.

[24] Cheng, Z., Jiang, Y., Mandon, E.C. and Gilmore, R. (2005) Identification of cytoplasmic residues of Sec61p involved in ribosome binding and cotranslational translocation. J. Cell Biol. 168, 67–77.

[25] Angelini, S., Deitermann, S. and Koch, H.G. (2005) FtsY, the bacterial signal-recognition particle receptor, interacts functionally and physically with the SecYEG translocon. EMBO Rep. 6, 476–481.

[26] Koch, H.G. and Muller, M. (2000) Dissecting the translocase and integrase functions of the Escherichia coli SecYEG translocon. J. Cell Biol. 150, 689–694.

[27] Shimohata, N., Nagamori, S., Akiyama, Y., Kaback, H.R. and Ito, K. (2007) SecY alterations that impair membrane protein folding and generate a membrane stress. J. Cell Biol. 176, 307–317.

[28] Menetret, J.F., Hegde, R.S., Heinrich, S.U., Chandramouli, P., Ludtke, S.J., Rapoport, T.A. and Akey, C.W. (2005) Architecture of the ribosome-channel complex derived from native membranes. J. Mol. Biol. 348, 445–457.

[29] Menetret, J.F., Neuhof, A., Morgan, D.G., Plath, K., Radermacher, M., Rapoport, T.A. and Akey, C.W. (2000) The structure of ribosome-channel complexes engaged in protein translocation. Mol. Cell 6, 1219–1232.

[30] Morgan, D.G., Menetret, J.F., Neuhof, A., Rapoport, T.A. and Akey, C.W. (2002) Structure of the mammalian ribosome-channel complex at 17A resolution. J. Mol. Biol. 324, 871–886.

[31] Beckmann, R., Spahn, C.M., Eswar, N., Helmers, J., Penczek, P.A., Sali, A., Frank, J. and Blobel, G. (2001) Architecture of the protein-conducting channel associated with the translating 80S ribosome. Cell 107, 361–372.