Subunit interactions in the *Escherichia coli* protein translocase: SecE and SecG associate independently with SecY

Takayuki Homma, Tohru Yoshihisa[1], Koreaki Ito*

Institute for Virus Research, Kyoto University, Kyoto 606-01, Japan

Received 14 March 1997

Abstract We used hexahistidine-tagged SecE and SecY to study how the core subunits (SecY, SecE and SecG) of *Escherichia coli* protein translocase interact with each other. Detergent extracts were prepared from the plasma membranes and fractionated by Ni^{2+}-NTA agarose affinity binding. Although His_6-SecE, expressed in wild-type cells, brought down both SecY and SecG, neither of them was brought down when the same protein was expressed in the *secY24* mutant cells. His_6-SecY brought down both SecE and SecG, as expected. Interestingly, His_6-SecY24 was able to bring down SecG but not SecE. These results confirm our previous conclusion that the *secY24* alteration impairs the SecY-SecE interaction, and demonstrate that SecY and SecG can form a complex that does not contain SecE. Likewise, SecY-SecE complex could be isolated from the *secG*-deleted strain. The trimeric complex, in detergent extracts, dissociated at a critical temperature between 23 and 26°C, whereas the SecY-SecE complex without SecG dissociated at a slightly lower temperature (20–23°C). We conclude that each of SecE and SecG independently binds to SecY, the central subunit of protein translocase, although the trimeric complex is more stable than the binary complexes.

Key words: Protein translocation; Protein-protein interaction; Membrane protein; Histidine tag; SecYEG; (*Escherichia coli*)

1. Introduction

The core component of protein translocation machinery in the plasma membrane of *Escherichia coli* consists of three integral membrane proteins, SecY, SecE and SecG [1,2]. Similar trimeric membrane protein complexes (Sec61 complexes) are also found in the endoplasmic reticulum membrane of yeast and mammalian cells [3]. One central question about these complexes is how these proteins interact with each other to constitute a transmembrane pathway for translocation of polypeptides. SecY, SecE and SecG of *E. coli* are multi-spanning membrane proteins with 10, 3 and 2 transmembrane segments, respectively [4–6]. Biochemical evidence for the SecYEG trimeric complex includes chromatographic co-elution of SecY, SecE and SecG after solubilization of the membrane with non-ionic detergents [7,8], and their co-immunoprecipitation with appropriate antibodies [8,9]. Whenever SecY is synthesized over SecE in vivo, the excess fraction is rapidly degraded by the FtsH proteolytic system, while the other fraction remains stable due presumably to the association with SecE [10–13]. SecY-SecE complex, once formed, does not seem to dissociate [9,11] (unlike an earlier proposal; [14]), whereas SecG interaction with the SecY-SecE complex may be more dynamic [9]. Indeed, SecG was shown to undergo striking topology inversion [15], which presumably is coupled with the SecA insertion/deinsertion events [16].

Genetic evidence also suggests that SecY and SecE are interacting functionally. 'Suppressor-directed inactivation' of SecY titrates out SecE, and synthetic lethal combinations of *secY* and *secE* mutations are known to exist [14,17]. A dominant negative variant of SecY, $SecY^{-d}1$, is thought to sequester SecE, since it is suppressible by overproduction of SecE [18]. It is also suppressible by intragenic suppressor mutations in cytoplasmic domain 4 (C4), suggesting that this domain of SecY is important for the SecY-SecE interaction [19]. Evidence suggests that the *secY24* amino acid substitution in C4 impairs the SecY-SecE interaction [19]. Importance of the second cytoplasmic segment (C2) of SecE for SecY-SecE interaction was suggested by mutational studies [20]. A binding between C2 of SecE and C4 of SecY may thus be suggested. Specific interactions were also proposed between transmembrane segment 10 of SecY and transmembrane segment 3 of SecE, as well as between periplasmic domain 1 of SecY and periplasmic domain 2 of SecE [21].

In contrast to the extensive information available for the SecY-SecE interaction, little is known about how SecG interacts with the other subunits. Nishiyama et al. [22] suggested, based on the observation that overproduced SecG stabilized a C-terminal fragment of SecE, that SecG primarily interacts with SecE. On the other hand, Bost and Belin [23] reported genetic suppression of some mutations of *secG* by those of *secY*. In the present work, we exploited the hexahistidine tagging technique [24] to study subunit interactions of protein translocase. Our results indicate that SecY serves as the central subunit of the trimer, to which SecE and SecG independently bind.

2. Materials and methods

2.1. E. coli strains and plasmids

AD202 was an *ompT*::*kan* [25] derivative of MC4100 [26] and AD206 additionally carried *secY24*, *rspE* and *zhd-33*::Tn*10* mutations [27]. TYE280 was a *recA cya* derivative of AD202 into which $F'lacI^Q$ $lacZ^-$ Y^+ had been introduced. THE494 was a Δ*secG*::*kan* [6] derivative of AD179 (MC4100, Δ*ompT*) [25] and constructed by P1 transduction. The *ompT* mutation was necessary to avoid artificial cleavage of SecY after cell disruption and membrane solubilization [25].

Plasmid pTYE024, a pACYC184-based plasmid with chloramphenicol resistance marker, carried his_6-*secE* under the *lac* promoter control. A 472 bp DNA fragment including the *secE* open reading frame with a 5′ attachment of 5′AGGAAAAAAGCATGCATCACCAT-

*Corresponding author. Fax: (81) 75-771-5699/(81) 75-761-5626.
E-mail: kito@virus.kyoto-u,ac.jp

[1]*Present address:* Department of Chemistry, Graduate School of Science, Nagoya University, Nagoya 464, Japan.

PII S0014-5793(97)00376-1

CACCATCA3′ (an artificial SD sequence for the translation that initiates as Met-His_6) was originally prepared by polymerase chain reaction, cloned into pBluescript SK(−), excised by *Sac*I and *Hin*dIII and finally cloned into *Sac*I and *Hin*dIII digested pSTV28 that had been subjected to *Eco*RI digestion, filling-in with Klenow DNA polymerase and ligation. pSTV28 was identical with pSTV29 [28] except that the multi-cloning site was inverted, and the *Eco*RI frame shifting was necessary to avoid an in-frame connection of His_6-SecE to the N-terminus of LacZ. pTYE017 was similar to pTYE024 except that the vector used was pUC118.

pTYE067, a pBR322-based plasmid with ampicillin resistance marker, carried *his*$_6$-*secY* under the *lac* promoter control. A double stranded oligonucleotide with a sequence 5′GAATTCTCACCATCACCATCACCATATCGAAGGTCGGGATCC3′ was inserted between the *Eco*RI and *Bam*HI sites of vector pTWV228 (obtained from Takara Shuzo) allowing an in-frame insertion of a His_6-Ile-Glu-Gly-Arg sequence into the LacZ sequence corresponding to the multi-cloning region. This vector was named pTYE057. The *secY* open reading frame and the following vector region were amplified from pKY248 [11] using an upstream primer with a 5′ extension of GGATCCG to the first nucleotide of *secY* and a down stream primer of a vector sequence. The product was digested with *Bam*HI and cloned into pTYE057, yielding a plasmid named pTYE064, whose sequence was confirmed for the *his*$_6$-*secY* 5′ region. Any PCR error was eliminated by replacing the *Sma*I-*Xba*I fragment of pTYE064 with the corresponding fragment of pKY318 [11]. The His_6-SecY protein encoded by the final product (pTYE067) contained an N-terminal extension of Met-Ile-Thr-Asn-Ser-His_6-Ile-Glu-Gly-Arg-Asp-Pro. It was necessary to use host cells carrying the *lacI*^{Q} mutation or medium containing glucose, to manipulate, propagate and maintain pTYE067, since His6-SecY overproduction was toxic. Plasmid pTYE083 was identical to pTYE067 except that it carried the *secY24* mutation [27]. For its construction, a 833 bp *Stu*I-*Eag*I fragment of pTYE067 was replaced by the corresponding fragment of pKY132 [19].

2.2. Media

L medium contained 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl and 1.7 mmol NaOH per liter. M9 medium [29] was supplemented with 2 μg/ml of thiamine, amino acids (20 μg/ml, other than Met and Cys) and either 0.4% glucose or 0.4% glycerol. Ampicillin (50 μg/ml) and chloramphenicol (20 μg/ml) were included for growing plasmid-bearing strains, as required.

2.3. Affinity isolation of the SecYEG complex

Cells, in which an appropriate His_6-tagged protein was expressed from a plasmid, were treated with EDTA-lysozyme-sucrose [30]. Spheroplasts thus formed were pelleted, resuspended in 50 mM HEPES-KOH (pH 7.0) containing 50 mM NaCl, 20% glycerol, 10 mM β-mercaptoethanol and 0.2 mM phenyl-methyl sulfonylfluoride, and disrupted by sonication. After removal of cell debris by low-speed centrifugation, supernatant was centrifuged (Beckman type 70.1Ti rotor, at 38 000 rpm for 1 h at 4°C) to obtain membrane pellets, which were then suspended in 50 mM HEPES-KOH (pH 8.0) containing 0.3 M NaCl, 20% glycerol, 10 mM β-mercaptoethanol and 0.2 mM phenyl-methyl sulfonylfluoride. The membrane suspension was then solubilized by addition of Triton X-100 to a final concentration of 1% and incubation at 0°C for 30 min. After removal of insoluble materials by centrifugation (Hitachi RP100-AT4 rotor at 60 000 rpm for 30 min at 4°C), supernatant was applied to a Ni^{2+}-NTA agarose spin column, which was washed 3× with 3 column vols. of the same buffer but containing 0.1% Triton X-100 and 10 mM imidazole by centrifuging at 800 rpm for 1 min at 4°C (Tomy MRX-150 microcentrifuge), and then eluted with 2 column vols. of 250 mM imidazole in the same buffer by centrifuging at 2000 rpm for 2 min at 4°C. Elution was repeated twice. Portions of fractions obtained were examined by SDS polyacrylamide gel electrophoresis (PAGE) and immunoblotting using anti-SecY [28], anti-SecE [10] and anti-SecG [6].

3. Results

3.1. His$_6$-SecE and His$_6$-SecY can be used to isolate SecYEG complex

We constructed plasmids pTYE024 and pTYE067 that encoded His_6-SecE and His_6-SecY, respectively. In these constructions, a sequence including hexahistidine was attached to the N-terminus of each protein. pTYE024 complemented the cold sensitivity of the *secE501* mutant [5], and pTYE067 (without full induction) complemented the temperature sensitive *secY24* mutant [27] as well as several cold-sensitive *secY* mutants [31]. Thus, neither the SecE function nor the SecY function was interfered with by the attachment of the histidine tag. When His_6-SecE is expressed, it will accumulate in the cell, and SecE-interacting components will bind either to the chromosomally encoded SecE molecules or to the His_6-SecE molecules. Since SecY is unstable without association with SecE, His_6-SecY is expected to enter the kinetic competition between degradation and association with SecE [10,11]. In binding to SecE, it should compete with the chromosomally encoded SecY molecules [18,19].

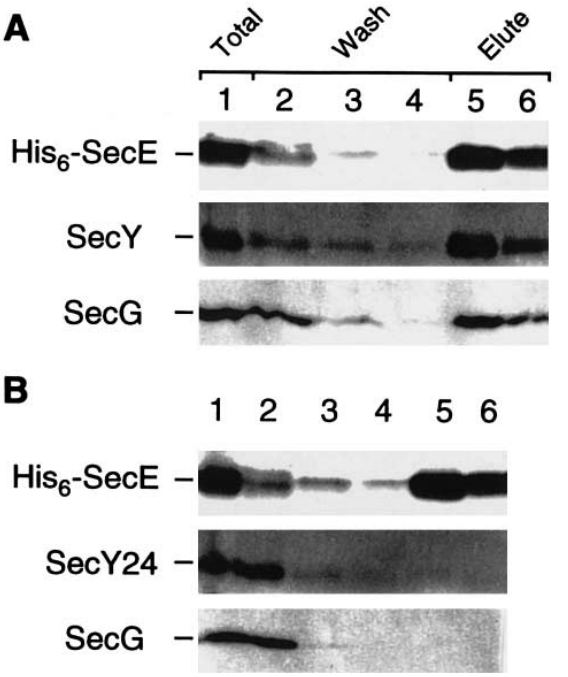

Fig. 1. Co-isolation of SecY and SecG with His_6-SecE. Plasmid pTYE024 (His_6-SecE) was introduced into AD202 (A: *secY*$^+$ *ompT*::*kan*) and AD206 (B: *secY24 ompT*::*kan*), and transformants were grown at 30°C in M9-glycerol medium. Membrane fractions were solubilized with Triton X-100 and subjected to Ni^{2+}-NTA agarose-affinity fractionation. Proteins in each fraction were separated by SDS PAGE and SecE, SecY and SecG (as indicated) were stained immunologically. Lane 1, total membrane extract (amount used was 1/12 the other fractions); lanes 2–4, washate fractions; lanes 5–6, imidazole eluate fractions.

Membrane fractions were prepared, solubilized with Triton X-100 and passed through Ni^{2+}-NTA agarose spin columns, which were washed and then eluted with the buffer containing imidazole. Each fraction was examined by SDS PAGE and immunoblotting for distribution of SecY, SecE and SecG. As shown in Fig. 1A (lanes 5–6), His_6-SecE was eluted by imidazole along with SecY and SecG. Likewise, as shown in Fig. 2A (lanes 6–7), elution of His_6-SecY was accompanied by co-elution of SecE and SecG. None of SecY, SecE or SecG was eluted by imidazole without the expression of a His_6-tagged protein (data not shown). These results show that SecY-SecE-SecG complex can be isolated conveniently by attaching a

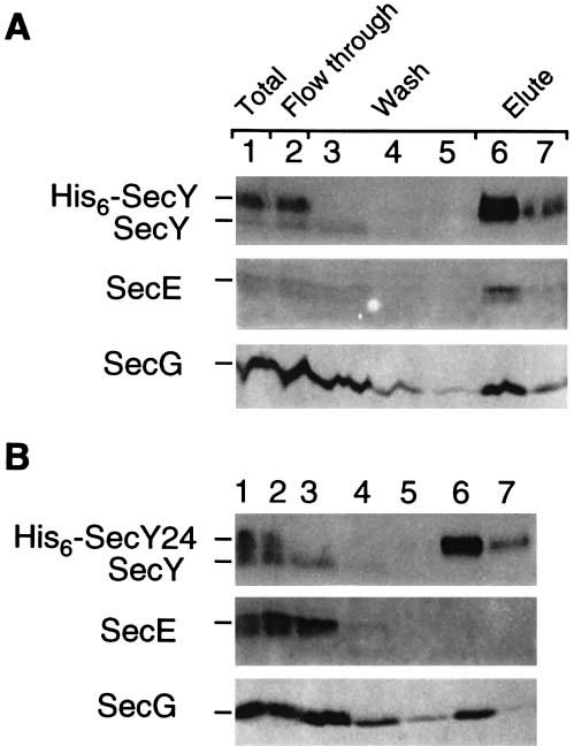

Fig. 2. Co-isolation of SecE and SecG with His_6-SecY. Plasmid pTYE067 (A: His_6-SecY) or pTYE083 (B: His_6-SecY24) was introduced into strain TYE280, and transformants were grown at 30°C in L-glucose medium. After induction with 0.1 mM isopropyl β-D-thiogalactoside (IPTG) and 0.1 mM cAMP for 40 min, membrane fractions were prepared and solubilized with Triton X-100. Samples were subjected to Ni^{2+}-NTA agarose-affinity fractionation and analyzed as described in the legend to Fig. 1. Lane 1, total membrane extract (amount used was 1/12 those used for lanes 3–7); lane 2, flow-through fraction (amount used was 1/5 those used for lanes 3–7); lanes 3–5, washate fractions; lanes 6–7, imidazole eluate fractions.

His_6 tag to either SecE or SecY and expressing the tagged protein in vivo.

Immunoblotting using antibodies against other Sec factors failed to detect SecA, SecD or SecF in the eluate fractions (data not shown). Syd [28] was detected in the eluates from the His_6-SecY or SecY24-containing samples, but not from the His_6-SecE-containing samples (data not shown). It was previously suggested that Syd is peripherally associated with the membrane, possibly via SecY [28].

3.2. Use of the SecY24 variant for demonstration of a SecY-SecG interaction

When His_6-SecE was expressed in the *secY24* mutant cells grown at 30°C (permissive temperature for this mutant), neither the altered SecY protein (SecY24) nor the SecG protein was co-eluted with it (Fig. 1B). This is consistent with our previous conclusion that the *secY24* mutation destabilizes the SecY-SecE complex [19]. The failure to obtain co-eluted SecG raises two possibilities: (1) the *secY24* mutation somehow interferes with SecE-SecG interaction; or (2) SecG is kept in the complex through its binding to SecY, rather than to SecE. To discriminate between these possibilities, we constructed a plasmid encoding His_6-SecY24 and expressed it in the wild-type cells. It was shown that His_6-SecY24 brought down SecG but not SecE (Fig. 2B). Thus, the *secY24* mutation selectively destabilizes the SecY-SecE interaction. Since SecY-SecG complex, without SecE, can be isolated, SecG independently binds to SecY.

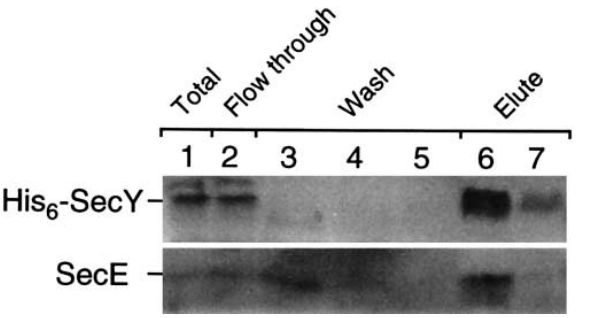

Fig. 3. SecY-SecE complex in the absence of SecG. Plasmid pTYE067 (His_6-SecY) was introduced into strain THE494 (Δ*secG* Δ*ompT*), and transformants were grown at 37°C in L-glucose medium. Affinity isolation of His_6-SecY and detection of co-isolated proteins were carried out as described in the legend to Fig. 2.

3.3. SecE binding to SecY is independent of SecG

To examine whether SecY-SecE complex can be isolated in the absence of SecG, we introduced the plasmid encoding His_6-SecY into a strain deleted for *secG* [6]. SecE was eluted with His_6-SecY (Fig. 3). Co-elution of SecY was also observed when His_6-SecE was expressed in the same strain (data not shown). Thus, a SecY-SecE complex can be formed in the absence of SecG.

3.4. Thermal instability of the complex in detergent extracts

Brundage et al. [8] reported that the co-immunoprecipitation of SecYEG complex was abolished when the detergent extract was incubated at temperatures of higher than ~20°C. We pre-incubated a detergent extract from the His_6-SecE-containing membrane at various temperatures and examined subsequent co-elution of SecY and SecG with His_6-SecE. As shown in Fig. 4, co-elution of both proteins was observed when the extract was pre-incubated up to at 23°C, but not at 26°C or higher. These results indicate that the SecE-SecY association in the detergent-solubilized state can only persist at low temperatures, and there is a critical temperature, between 23 and 26°C, where the complex dissociates. To examine whether SecY-SecG association is also temperature-labile, similar experiment was carried out using His_6-SecY. Co-elu-

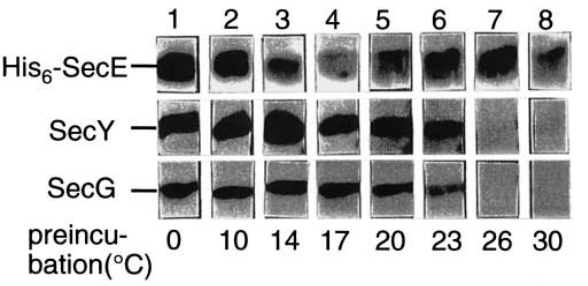

Fig. 4. Thermal instability of the SecY-SecE association in detergent-solubilized states. Cells of AD202 carrying pTYE024 (His_6-SecE) were grown at 37°C in M9-glycerol medium and a Triton X-100 extract of membrane was pre-incubated at the temperatures indicated for 30 min, which was then subjected to Ni^{2+}-NTA agarose fractionation at 4°C. Only the first eluate fractions are shown.

tion of SecG with His_6-SecY was also abolished at a temperature between 23 and 26°C (Fig. 5). Thus, both the SecY-SecE interaction and the SecY-SecG interaction are labile at ambient temperatures. Temperature profiles of the SecY-SecE association was affected somewhat by the presence or absence of SecG. In the absence of SecG, the SecY-SecE complex dissociated by pre-incubation at 23°C (Fig. 6). Thus, the presence of SecG stabilizes the SecY-SecE association to a small extent (by ≈3°C in terms of the critical dissociation temperature).

4. Discussion

The SecYEG trimeric complex in the detergent-solubilized state is unstable as manifested by its dissociation at a temperature between 23 and 26°C. This behavior of the solubilized complex does not seem to correlate with the stability of the SecYEG complex in vivo, since protein export in vivo is active at temperatures as high as 37–42°C and no SecY/SecE subunit exchange occurs [9]. Instead, protein export in vivo may include an intrinsically cold-sensitive step [32]. Pohlschröder et al. [20] reported that a number of *secE* mutations abolish SecE-SecY co-immunoprecipitation even when they affected protein export only slightly or negligibly. Although the present experimental system has the limitation just discussed, detection of co-elution positively indicates the existence of subunit interaction. We were able to obtain a SecY-SecG complex that did not contain SecE. This was made possible by using the *secY24* mutant form of SecY. We were also able to obtain a SecY-SecE complex that did not contain SecG, using the *secG* deletion strain. These results clearly indicate that SecY is the central subunit of the translocase complex to which SecE and SecG bind independently. However, the pre-incubation results suggest that the presence of all the three subunits is necessary for the maximum stability.

The conclusion stated above is at variance with the conclusion of Nishiyama et al. [22] who proposed that SecG primarily binds to SecE. Although our results by no means exclude a SecG-SecE interaction, the SecG-dependent increase in accumulation of a truncated SecE fragment [22] only indirectly suggests their mutual interaction. Our results (Fig. 1B) suggest that SecE-SecG interaction, if any, is not sufficiently strong to survive the detergent extraction and the affinity isolation conditions used in the present experiments.

Although the SecY24 mutant protein is impaired in the SecY-SecE interaction, we believe that even this mutant protein is complexed with SecE in vivo at least at 30°C, since it is

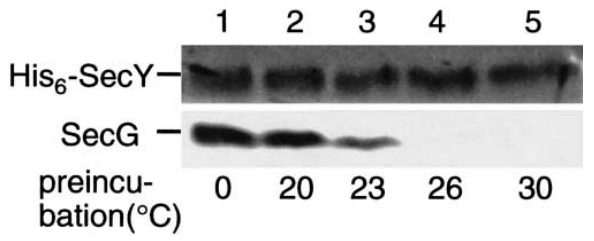

Fig. 5. Thermal instability of the SecY-SecG association in detergent-solubilized states. Cells of TYE280 carrying pTYE067 (His_6-SecY) were grown at 37°C in L-glucose medium and induced with 1 mM IPTG and 1 mM cAMP for 40 min. A Triton X-100 extract of membrane was pre-incubated at the temperatures indicated for 30 min, and subjected to Ni^{2+}-NTA agarose fractionation 4°C. Only the first eluate fractions are shown.

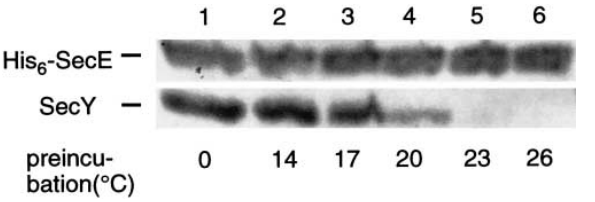

Fig. 6. Thermal stability of the SecY-SecE association in the absence of SecG. Cells of THE494 (Δ*secG* Δ*ompT*) carrying pTYE017 (His_6-SecE) were grown at 37°C in L-glucose medium. A Triton X-100 extract of membrane was pre-incubated at the temperatures indicated for 30 min, and subjected to Ni^{2+}-NTA agarose fractionation at 4°C. Only the first eluate fractions are shown.

functional and stable at this permissive temperature [19]. Furthermore, the suppression of the temperature sensitivity by a proteolysis-defective mutation in *ftsH* suggests that even at 42°C the SecY24 protein can function and, hence, can interact with SecE [12]. It is most likely that after solubilization in the detergent solution, the SecY24-SecE complex dissociates more readily than the wild-type complex.

Although His_6-SecY and SecY were separable in gel electrophoresis, we observed no detectable co-elution of the chromosomally encoded SecY with plasmid-encoded His_6-SecY (Fig. 2). Thus, a SecYEG trimer complex may contain only one molecule of SecY. However, we consistently observed that SecY tailed into the washate fraction (Fig. 2A, lane 3). This could represent a weak interaction of SecY with the His6-SecY complex. Recently, Hanein et al. [33] observed, by electron microscopy, an oligomeric assembly of the Sec61 core complexes in yeast and dog pancreas. It will be tempting to study whether the SecYEG trimeric complexes can further oligomerize into a suprastructural unit.

Acknowledgements: We thank H. Tokuda and K. Nishiyama (University of Tokyo) for the original Δ*secG* strain as well as for anti-SecG serum, Y. Akiyama, H. Mori and E. Matsuo for their stimulating discussion, and K. Mochizuki for laboratory supplies. This work was supported by grants from The Ministry of Education, Science and Culture, Japan, and from Human Frontier Science Program Organization.

References

[1] W. Wickner, M.R. Leonard, J. Biol. Chem. 271 (1996) 29514–29516.
[2] K. Ito, Genes Cells 1 (1996) 337–346.
[3] B. Jungnickel, T.A. Rapoport, E. Hartmann, FEBS Lett. 346 (1994) 73–77.
[4] Y. Akiyama, K. Ito, EMBO J. 6 (1987) 3465–3470.
[5] P.J. Schatz, P.D. Riggs, A. Jacq, M.J. Fath, J. Beckwith, Genes Dev. 3 (1989) 1035–1044.
[6] K. Nishiyama, M. Hanada, H. Tokuda, EMBO J. 13 (1994) 3272–3277.
[7] L. Brundage, J.P. Hendrick, E. Schiebel, A.J.M. Driessen, W. Wickner, Cell 62 (1990) 649–657.
[8] L. Brundage, C.J. Fimel, S. Mizushima, W. Wickner, J. Biol. Chem. 267 (1992) 4166–4170.
[9] J.C. Joly, M.R. Leonard, W.T. Wickner, Proc. Natl. Acad. Sci. USA 91 (1994) 4703–4707.
[10] S. Matsuyama, J. Akimaru, S. Mizushima, FEBS Lett. 269 (1990) 96–100.
[11] T. Taura, T. Baba, Y. Akiyama, K. Ito, J. Bacteriol. 175 (1993) 7771–7775.
[12] A. Kihara, Y. Akiyama, K. Ito, Proc. Natl. Acad. Sci. USA 92 (1995) 4532–4536.

[13] Y. Akiyama, A. Kihara, H. Tokuda, K. Ito, J. Biol. Chem. 271 (1996) 31196–31201.
[14] K. Bieker-Brady, T.J. Silhavy, EMBO J. 9 (1992) 3165–3174.
[15] K. Nishiyama, T. Suzuki, H. Tokuda, Cell 85 (1996) 71–81.
[16] A. Economou, W. Wickner, Cell 78 (1994) 835–843.
[17] K.L. Bieker, T.J. Silhavy, Cell 61 (1990) 833–842.
[18] T. Shimoike, Y. Akiyama, T. Baba, T. Taura, K. Ito, Mol. Microbiol. 6 (1992) 1205–1210.
[19] T. Baba, T. Taura, T. Shimoike, Y. Akiyama, T. Yoshihisa, K. Ito, Proc. Natl. Acad. Sci. USA 91 (1994) 4539–4543.
[20] M. Pohlschröder, C. Murphy, J. Beckwith, J. Biol. Chem. 271 (1996) 19908–19914.
[21] A.M. Flower, R.S. Osborne, T.J. Silhavy, EMBO J. 14 (1995) 884–893.
[22] K. Nishiyama, S. Mizushima, H. Tokuda, Biochem. Biophys. Res. Commun. 217 (1995) 217–223.
[23] S. Bost, D. Belin, EMBO J. 14 (1995) 4412–4421.
[24] R. Gentz, C.H. Chen, C.A. Rosen, Proc. Natl. Acad. Sci. USA 86 (1989) 821–824.
[25] Y. Akiyama, K. Ito, Biochem. Biophys. Res. Commun. 167 (1990) 711–715.
[26] M.J. Casadaban, J. Mol. Biol. 104 (1976) 541–555.
[27] K. Shiba, K. Ito, T. Yura, D.P. Cerretti, EMBO J. 3 (1984) 631–635.
[28] T. Shimoike, T. Taura, A. Kihara, T. Yoshihisa, Y. Akiyama, K. Cannon, K. Ito, J. Biol. Chem. 270 (1995) 5519–5526.
[29] Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[30] I. Yamato, Y. Anraku, K. Hirosawa, J. Biochem. 77 (1975) 705–718.
[31] T. Taura, Y. Akiyama, K. Ito, Mol. Gen. Genet. 243 (1994) 261–269.
[32] K.J. Pogliano, J. Beckwith, Genetics 133 (1993) 763–773.
[33] D. Hanein, K.E.S. Matlack, B. Jungnickel, K. Plath, K.-U. Kalies, K.R. Miller, T.A. Rapoport, C.W. Akey, Cell 87 (1996) 721–732.