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Roles of the Conserved Cytoplasmic Region and Non-Conserved Carboxy-Terminal Region of SecE in *Escherichia coli* Protein Translocase[1]

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SecE, an essential membrane component of the *Escherichia coli* protein translocase, consists of 127 amino acid residues. Only a part of the second putative cytoplasmic region comprising some 13 residues is essential for the SecE function as long as the proper topological arrangement is retained. The Trp84 and Pro85 residues of this region are conserved in all eubacterial SecE homologues. The conservation of positively charged residues corresponding to Arg80 and Lys81 is also substantial. We deleted or replaced these residues to assess their roles in the SecE function. Deletion of the Arg80-Lys81 dipeptide did not abolish the SecE function whereas that of Trp84 or Pro85 caused a loss of the function. Strikingly, however, replacement of Pro85 with either Gly, Ser, or Ala, and that of Trp84 with Lys did not abolish the SecE function. These results indicate that the strong conservation of these residues does not reflect their obligatory requirement for the SecE function. A chimeric SecE possessing the cytoplasmic region of the *E. coli* SecE and the following region of the *Bacillus subtilis* SecE was able to form the translocation machinery together with SecA, SecY, and SecG. Although a Leu to Arg mutation at position 108 has been thought to cause a loss of signal recognition fidelity and thereby suppress a signal sequence defect, the same mutation at position 111 caused a complete loss of the function. The levels of SecY and SecG in the *secEcsE501* mutant, which expresses SecE at a decreased level and is sensitive to low temperature, increased upon the expression of functional SecE derivatives, irrespective of the site of mutation, suggesting that the levels of SecY and SecG are co-operatively determined by the level of functional, but not non-functional, SecE. Based on these results, the SecE function in the translocase is discussed.

Key words: conserved residues, *Escherichia coli*, protein translocation, SecE, SecY.

The involvement of various Sec factors in protein translocation across the cytoplasmic membrane of *Escherichia coli* has been examined both genetically and biochemically (*1-5*). Reconstitution of the protein translocation machinery from independently purified SecY, SecE, and SecA (*6*), or the SecY/SecE complex and SecA (*7*) has established the essential roles of these three factors in protein translocation. The SecY/SecE complex contained another protein, termed band 1 (*7, 8*). Moreover, a new membrane factor, SecG (p12), was found to stimulate protein translocation (*9, 10*). It was then revealed that SecG and band 1 are identical (*11*). Taken together, these results indicate that the central part of the translocase of *E. coli* comprises three membrane protein subunits, SecY, SecE, and SecG, and a peripheral factor, SecA (*12*).

The Sec61p complex, the protein translocation machinery in the mammalian ER membrane, also comprises three protein subunits (*13*), including homologues of SecY and SecE (*14, 15*), suggesting the evolutionary conservation of components of the translocation machinery from prokaryotes to mammals. Various SecE homologues found in both eukaryotes and prokaryotes contain a single transmembrane segment and a preceding putative cytoplasmic region (*15, 16*), except for a SecE homologue of *Haemophilus influenza* (accession numbers, L42023 and HI0716). Although *E. coli* SecE contains three transmembrane segments (*17*), a truncated SecE derivative containing only the third transmembrane segment and a portion of the preceding cytoplasmic region has been shown to retain the SecE function (*18, 19*). The sequence of this cytoplasmic region is conserved among SecE homologues, whereas little similarity exists in the other regions (*15, 16, 20*). Replacement of the conserved cytoplasmic region, but not the third transmembrane segment, with a heterologous sequence has been shown to abolish the SecE function (*16*). These results indicate that the third transmembrane segment of SecE merely functions as a membrane-anchoring for the preceding conserved region, that is essential for the SecE function.

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Abbreviations: IPTG, isopropyl-β-D-thiogalactopyranoside; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; OmpA, outer membrane protein A; CBB, Coomassie Brilliant Blue.

On the other hand, PrlG (SecE) suppressors, that allow the secretion of proteins possessing defective signal peptides, have been found to contain altered residues in the third transmembrane segment or following periplasmic region (*18, 21*). Furthermore, *in vivo* genetic studies on synthetic lethality between the *prlA* (*secY*) and *prlG* (*secE*) alleles suggested that the third transmembrane segment of SecE interacts with the tenth transmembrane segment of SecY (*22-24*). These observations suggest that alteration of the third transmembrane and following periplasmic regions, although no essential residue has been found in these regions, affects the signal recognition fidelity of the translocation machinery.

Recent genetic analysis of SecE by Murphy and Beckwith (*16*) revealed that the sequence starting from Glu78 to Thr90 in the conserved cytoplasmic region is crucial for the SecE function. Among the 13 residues in this sequence, the Trp-Pro dipeptide is conserved in all known eubacterial SecE homologues. Furthermore, the dipeptide is also present in Syd, which was reported to interact with and stabilize SecY (*25*). The Pro residue, an α-helix breaker, is present in the middle of the conserved cytoplasmic region, and may have special importance for the structure of the conserved region.

To assess the roles of the conserved residues, we constructed various derivatives of SecE, and examined their activities both *in vivo* and *in vitro*. We found that the conserved residues are not essential for the SecE function.

EXPERIMENTAL PROCEDURES

Bacterial Strains—*E. coli* TG1 (*26*), PR520 (MC4100 *secEcsE501 argE::Tn10*) (*27*), PS274 (KS272 *secEΔ19-111* pJS65) (*18*), and SM7000 (W3110 *ompT secEcsE501 argE::Tn10*) (*19*) were used.

DNA Manipulations—SecE-C derivatives shown in Fig. 1 were constructed on the DNA level by PCR as described (*28*). The oligonucleotide primer which anneals at the 5′ ends of the *secE-C* derivatives (5′ terminal primer) was always the same; 5′ CAAGGATCCTAGGAGGTTTAAATTTATG 3′. The primers which anneal at the 3′ ends of the genes (3′ terminal primers) were as follows: 5′ CAACTCGAGTCAGAACCTCAGGCCAGTG 3′ (*Δ*WP and *Δ*RK derivatives), 5′ CAACTCGAGAGGACTGTCCAGTCCTTCC 3′ (P*Bs*), and 5′ CAAGTCGACTTAGAACCTCAGGCCAGTGA [*Δ*P, P(G), P(S), P(A), *Δ*W, and W(K)]. pMAN-809 carrying the *E. coli secE* (*29*), pE1 carrying *secE-C* (*19*), and pTE814R carrying the *Bacillus subtilis secE* (*20*) were used as templates for PCR. A PCR fragment containing the coding region of each of the *secE-C* derivatives from the 5′ end to the fusion or deletion site (5′ fragments) was amplified with the 5′ terminal primer and an appropriate adapter primer (adapter 1). The adapter oligonucleotides were designed to create the mutations. The 3′ fragment of each of the *secE-C* derivatives was amplified with the 3′ terminal primer and the adapter 2 primer, the latter of which comprises a 34-mer and is complementary to adapter 1. The 3′ fragments of P*Ec*1 and R-P*Ec*1 were synthesized with a DNA synthesizer. The amplified 5′ and 3′ fragments were purified and combined to form a double strand at the adapter region. The single-stranded region was filled in with AmpliTaq using a DNA thermal cycler (Perkin Elmer Cetus). Terminal primers were then added and fragments corresponding to the whole coding regions of the *secE-C* derivatives were amplified.

The PCR generated genes were next digested with *Bam*H1 and *Xho*I or *Sal*I, followed by ligation with pUSI2 that had been cut with *Bam*HI and *Sal*I. pUSI2 carries the *lacI* gene and *tac* promoter (*30*). *E. coli* strain TG1 or SM7000 was transformed with the ligated DNA and ampicillin-resistant transformants were screened for the inserted DNA. Finally, these constructs were confirmed by sequencing of the respective fragments.

Complementation of secE Mutations—The ability of *secE-C* derivatives to complement the *secEcsE501* mutation was examined as described (*19*). Complementation of *secEΔ19-111* was examined as described (*18*).

In Vitro Protein Translocation—Everted membrane vesicles were prepared as described (*31*) from PS274 cells harboring a plasmid, which carried the gene for either SecE-C, P(S), P(G), P(A), or W(K), and examined for the translocation of ^{35}S-labeled proOmpA D26, a truncated derivative of proOmpA (*32*), at 37°C as described (*33*). The assay was carried out in 50 mM potassium phosphate (pH 7.5) containing, 2 mM ATP, 2 mM $MgSO_4$, and 2.5 μg of membrane vesicles, with or without 10 μM carbonylcyanide *m*-chlorophenyl hydrazone.

Proteoliposomes were reconstituted with SecY, SecG, and SecE-C or P*Bs* as described (*12*). Translocation of [^{35}S]proOmpA D26 into the reconstituted proteoliposomes was assayed in the presence of SecA and ATP as described (*32*).

At specified times, an aliquot (25 μl) of the reaction mixture was withdrawn and treated with 0.8 mg/ml proteinase K on ice for 30 min. The translocated protein, which was resistant to proteinase K, was detected after SDS-

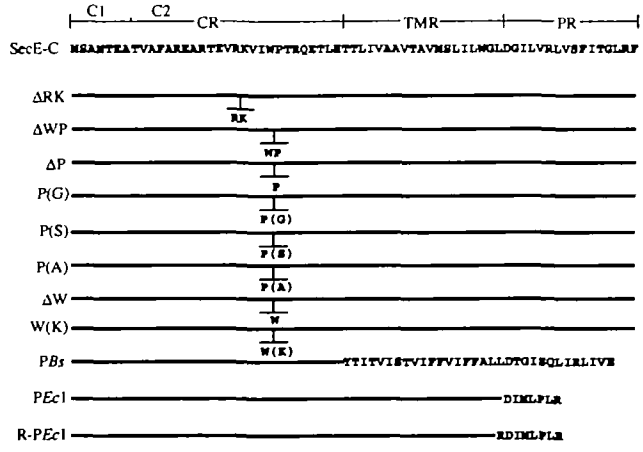

Fig. 1. **Structures of SecE-C derivatives.** The uppermost sequence represents SecE-C. The putative cytoplasmic (CR), transmembrane (TMR), and periplasmic (PR) regions are shown above the sequence. C1 and C2 represent partial sequences derived from the first and second cytoplasmic regions of SecE, respectively. Below SecE-C are shown the SecE-C derivatives, with the changed regions indicated by letters and the unchanged regions indicated by thick lines. The deletions and replacements of the conserved Arg(R), Lys(K), Trp(W), and Pro(P) residues are also shown. P*Bs* is a chimeric derivative comprising the cytoplasmic region from SecE-C and the following region from the *B. subtilis* SecE homologue. The periplasmic region of SecE-C was replaced by the first periplasmic sequence of SecE without (P*Ec*1) or with (R-P*Ec*1) Arg.

PAGE by means of fluorography as described (*34*). Densitometric quantification of band materials was carried out with a Shimadzu CS-930 chromatoscanner. The total amounts of proOmpA D26 and mature OmpA D26, which were translocated, were expressed as percentages of the input precursor protein. The numbers of methionine residues in proOmpA D26 (3 residues) and OmpA D26 (2 residues) were taken into consideration.

Purification of SecE-C and PBs—SecE-C was purified as described (*19*). The P*Bs* derivative was purified from *E. coli* cells overproducing this protein. Cytoplasmic membrane fractions prepared from these cells were treated with an equal volume of 10 M urea in 50 mM potassium phosphate (pH 6.0) at a protein concentration of 5 mg/ml.

For the purification of P*Bs*, urea-washed membranes were solubilized at a protein concentration of 5 mg/ml with 10 mM potassium phosphate (pH 6.0) containing 2.5% (w/v) octylglucoside, 10% (w/v) glycerol, and 10 mM $MgSO_4$ on ice for 10 min. The supernatant containing P*Bs* was applied on a MonoS HR 10/10 column (Pharmacia) that had been equilibrated with 10 mM potassium phosphate (pH 6.0) containing 10% (w/v) glycerol, 10 mM $MgSO_4$, and 2.5% octylglucoside. The column was developed at the flow rate of 4 ml/min with a linear gradient of 0-0.4 M NaCl in the same buffer. Fractions (2 ml) were analyzed by SDS-PAGE, followed by immunoblotting with an antibody raised against SecE. P*Bs* was eluted at about 0.3 M NaCl. Fractions containing P*Bs* were combined, concentrated, and then applied on a Superdex 75 HR 10/30 size-exclusion column that had been equilibrated with 50 mM potassium phosphate (pH 7.5) containing 10% (w/v) glycerol, 150 mM NaCl, and 2.5% octylglucoside. The column was eluted with the same buffer at the flow rate of 0.4 ml/ml. Fractions containing P*Bs* were combined and then concentrated to about 100 μg/ml.

Other Methods—SecY (*12*), SecG (*9*), and SecA (*35*) were purified as described. [^{35}S]proOmpA D26 was prepared as described (*34, 36*). SDS-PAGE and immunoblotting were performed as described (*19*). Antibodies were raised in rabbits against SecA, SecE, and the synthetic peptide corresponding to the Ser426 to Arg443 region of SecY or Gln95 to Asn110 region of SecG.

RESULTS AND DISCUSSION

Construction of SecE-C Derivatives—Various lines of evidence indicate that SecE and SecY interact with each other (*7, 12, 22, 29, 37, 38*), and exist as a complex, which also contains SecG (*7, 9, 11, 12*), in the cytoplasmic membrane. The cytoplasmic regions of SecE and SecY are involved in this interaction (*37*), although the transmembrane sequences may also contribute to the interaction (*23, 24*). Only a part of the second cytoplasmic region of SecE is essential for the function and conserved among various eubacterial SecE homologues (Fig. 2), whereas little similarity exists in the following transmembrane and periplasmic regions (*15, 16*). Therefore, it is plausible that the conserved region contains essential residues for the SecE function.

To assess the importance of the residues or domains, various derivatives of SecE-C were constructed (Fig. 1). SecE-C is a truncated derivative of SecE and retains the SecE function, although it comprises only a part of the second cytoplasmic region and the following transmembrane and periplasmic regions (*19*). The ability of these constructs to complement the cold-sensitive (*cs*) phenotype of the *secEcsE501* mutant, PR520 (*27*), and the chromosomal *secEΔ19-111* deletion mutant, PS274 (*18*), was then examined. The lower level of SecE than normal in *secEcsE501* causes the *cs* phenotype (*18*). The viability of the *secEΔ19-111* mutant, PS274, depends on plasmid pJS65 carrying *secE* (*18*). This plasmid also carries *kan*, which confers kanamycin-resistance. Plasmids carrying *secE-C* derivatives and *bla* were transformed into PS274 harboring pJS65. Ampicillin-resistant and kanamycin-sensitive transformants were then screened. These transformants should have lost plasmid pJS65, and *secE-C* derivatives should have taken on the complementing function of *secE* on pJS65.

Highly Conserved Trp-Pro Residues Are Replaceable—It has been reported that deletion of the Ala7-Glu78 region containing the N-terminal two transmembrane sequences does not abolish the SecE function, whereas the deletion of Glu78-Thr90 causes a loss of the function (*16*). Because of Glu at 6, the Δ7-78 derivative possesses Glu at the position corresponding to 78. Taken together, these results suggest that the sequence from Glu78 to Thr90 of SecE contains the essential region or residues for the function. Among the 13 residues in this region, positively charged residues corresponding to Arg80 and Lys81 are conserved among most SecE homologues (Fig. 2). However, plasmids carrying the gene for ΔRK complemented not only *secEcsE501* but also *secEΔ19-111*, indicating that the Arg-Lys residues or positive charges in this region are not essential for the SecE function (Table I). The Trp84-Pro85 dipeptide is conserved in all known eubacterial SecE homologues and Syd (Fig. 2). The deletion of either Trp (ΔW) or Pro (ΔP), or both (ΔWP) abolished the SecE function. We then substituted Trp with Lys [W(K)], and Pro with either Gly [P(G)], Ser

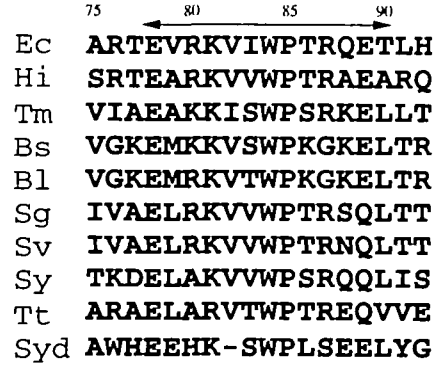

Fig. 2. **Alignment of the conserved cytoplasmic sequences of eubacterial SecE homologues.** Amino acid residues are expressed by the one letter code. Partial sequences of the putative cytoplasmic region of the SecE homologues are aligned as reported (*15, 16*). The sequence of the SecE homologue of *Haemophilus influenza* (accession numbers, L42023 and HI0716) is also given. The sequence of Syd, which interacts with and stabilizes SecY (*25*), is also shown. The uppermost numbers correspond to residues of *E. coli* SecE. The sequence from Glu78 to Thr90 (indicated by a line) is the minimum essential region for the function (see the text for details). Ec, *E. coli*; Hi, *H. influenza*; Tm, *Thermotoga maritima*; Bs, *Bacillus subtilis*; Bl, *B. licheniformis*; Sg, *Staphylococcus griseus*; Sv, *S. virisiniae*; Sy, *Synechocystis* sp.; Tt, *Thermus thermophilus*.

[P(S)], or Ala [P(A)]. Surprisingly, all the constructs complemented both *secEcsE501* and *secEΔ19-111* (Table I), indicating that both Trp and Pro residues can even be replaced with a dissimilar residue without a loss of function despite their high conservation.

In Vitro Translocation Activity of the Cytoplasmic SecE-C Derivatives—To determine whether or not replacement of the highly conserved Trp84 and Pro85 causes any difference in the translocation efficiency, *in vitro* protein translocation into membrane vesicles was examined. Membrane vesicles were prepared from the PS274 (*secEΔ19-111*) strain harboring plasmids, carrying the gene for either the P(G), P(S), P(A), or W(K) derivative, and then assayed for the translocation of proOmpA D26, a truncated derivative of proOmpA (*32*). The levels of these derivatives in membrane vesicles somewhat varied depending on the species of derivative (Fig. 3A), suggesting a difference in their stability. The translocation of proOmpA D26 into these membrane vesicles was assayed in the absence of the proton motive force (Fig. 3B). The proton motive force was abolished by the addition of carbonylcyanide *m*-chlorophenyl hydrazone since the membrane vesicles possessed F_0F_1-ATPase. The translocation activity supported by the derivatives was lower than that supported by SecE-C, indicating that the replacement of Trp84 or Pro85 impaired the SecE function to some extent. On the other hand, in the presence of the proton motive force, these derivatives supported the translocation as effectively as SecE-C does (data not shown). Impairment of the SecE function is therefore not serious in cells, which always generate a proton motive force. Taken together, these results indicate that replacement of the highly conserved Trp84 and Pro85 residues does not abolish the SecE function either *in vivo* or *in vitro*, whereas the deletion of Trp84 or Pro85 abolished the SecE function.

None of the four conserved residues thus examined was found to be obligatory. It should be noted, however, that as to the importance of Trp84 and Pro85, we only examined the replacement of one of these two residues at one time. It is also noteworthy that SecE derivatives, which were found to be functional at 37°C, may not be functional at a higher or lower temperature, as reported (*16*). Therefore, the functional SecE derivatives above may merely satisfy the minimum requirement for the function.

Roles of Non-Conserved Transmembrane and Periplasmic Regions—*B. subtilis* SecE has been reported to complement *secEcsE501* (*20*). Except in their cytoplasmic regions, no sequence similarity exists in the transmembrane and periplasmic regions between *E. coli* and *B. subtilis* SecEs. To examine a possible functional difference in the non-conserved regions, we constructed a chimeric derivative comprising the cytoplasmic region from SecE-C and the following region from *B. subtilis* SecE. This chimeric SecE-C derivative (P*Bs*) complemented both *secEcsE501* and *secEΔ19-111* (Table I). The difference in the activity between SecE-C and P*Bs* was further examined by means of reconstituted proteoliposomes. SecE-C and P*Bs* were purified and analyzed by SDS-PAGE, followed by Coomassie Brilliant Blue staining or immunoblotting (Fig. 4A). Only a single band was detected for both preparations. The purified protein was reconstituted into liposomes together with SecY in the presence and absence of SecG. The translocation of proOmpA D26 into the proteoliposomes was then assayed in the presence of SecA and ATP (Fig. 4B). The activity reconstituted with P*Bs* was higher than that with SecE-C when SecG was absent. The presence of

TABLE I. **Ability of plasmids encoding SecE-C derivatives to complement chromosomal *secE* mutations.**

Plasmid	SecE derivatives	Complementation	
		secEcsE501[a]	*secEΔ19-111*[b]
pUSI2	None	−	−
pE1	SecE-C	+	+
pJVK19	ΔRK	+	+
pJVK24	ΔWP	−	−
pJVK59	ΔW	−	−
pJVK55	ΔP	−	−
pJVK68	W(K)	+	+
pJVK64	P(G)	+	+
pJVK82	P(S)	+	+
pJVK84	P(A)	+	+
pJVK14	P*Bs*	+	+
pJVK48	P*Ec*1	+	+
pJVK44	R-P*Ec*1	−	−

[a]PR520 (*secEcsE501*) cells harboring the indicated plasmid were plated and then incubated at 20°C for 2 days in the presence of 1.5 mM IPTG. [b]The ability of the indicated plasmid to take over the complementing function of pJS65 carrying the *secE* gene was examined at 37°C.

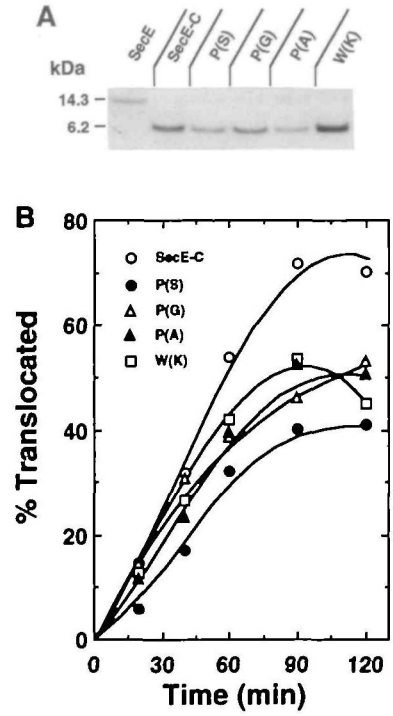

Fig 3 **Highly conserved Trp84 and Pro85 can be replaced without loss of the SecE function.** A: Everted membrane vesicles were prepared from PS274 (*secEΔ19-111*) cells harboring a plasmid encoding SecE (pJS65), SecE-C (pE1), P(S) (pJVK82), P(G) (pJVK64), P(A) (pJVK84), or W(K) (pJVK68). The levels of these derivatives in 20 μg of membrane vesicles were determined by SDS-PAGE, followed by immunoblotting with the anti-SecE antiserum. The migration positions of molecular weight markers are also shown. B: The translocation of ^{35}S-labeled proOmpA D26 into the everted membrane vesicles mentioned in A was assayed at 37°C in the absence of the proton motive force, as described under "EXPERIMENTAL PROCEDURES."

SecG caused remarkable stimulation of not only SecE-C-dependent activity but also P*Bs*-dependent activity. Taken together, these results indicate that the non-conserved regions of the two bacterial SecEs are functionally equivalent.

It has been reported that the replacement of the transmembrane region with a heterologous sequence did not abolish the SecE function (*16*). On the other hand, the mutation points of the four *prlG* (*secE*) alleles capable of suppressing signal sequence defects have been mapped in the transmembrane or periplasmic region, suggesting that these are hot regions affecting the ability of signal sequence recognition (*21*). These mutant proteins are thought to have lost the fidelity of signal recognition, thereby allowing the translocation of a precursor having a defective signal peptide. One of the *prlG* suppressors, *prlG1*, has a Leu to Arg mutation at position 108 (*18, 21*). On the other hand, the same Leu to Arg mutation at 111 was found to abolish the SecE function. We replaced the periplasmic sequence of SecE-C with the first periplasmic sequence, DIMLPLR, of SecE (*17*), by fusing this sequence to Leu111, and found that the derivative, P*Ec*1, was active (Table I). Since the first periplasmic sequence possesses Arg immediately before Asp, another replacement was made by fusing the RDIMLPLR sequence to Gly 110. Therefore, the construct, R-P*Ec*1, is a derivative of P*Ec*1 and possesses the Leu to Arg mutation at 111 (Fig. 1B). The R-P*Ec*1 derivative was inactive (Table I). These results suggest that Arg introduced around 108–111 significantly affects the SecE functions such as signal sequence recognition or support of the translocation. Both P*Ec*1 and R-P*Ec*1 possess the first periplasmic region instead of the second one (Fig. 1). Significance of the Arg introduction around 108–111 might be affected by this replacement.

Levels of SecA, SecG, SecY, and SecE-C Derivatives in the secEcsE501 Strain—The mutation in *secEcsE501* was mapped in the upstream region of the *secE* gene (*18*), and causes the expression of intact SecE at a decreased level at both 37 and 20°C (*9, 18*). The cold sensitive phenotype of this strain has been suggested to reflect the intrinsic cold sensitivity of protein translocation (*39*). A decrease in the level of SecE accompanies a decrease in the level of SecY (*9*) since the stable existence of SecY in the membrane requires the interaction with SecE (*29*). Furthermore, we

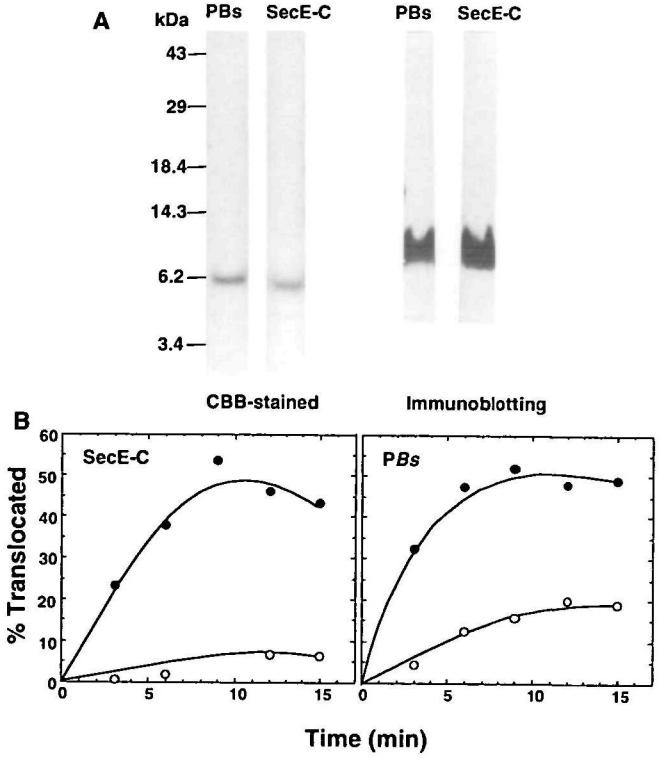

Fig. 4. **Non-conserved transmembrane and periplasmic regions of *E. coli* and *B. subtilis* SecEs are functionally equivalent.** A: SecE-C and P*Bs* were purified as described under "EXPERIMENTAL PROCEDURES" and then analyzed by SDS-PAGE, followed by either staining with Coomassie Brilliant Blue (CBB) or immunoblotting with the anti-SecE antibody. The amounts of protein applied on the gel were 1 μg for CBB-staining and 2 μg for immunoblotting. A large excess of protein was analyzed by immunoblotting to confirm that no intact SecE contaminated the samples. B: The purified SecE-C (15 μg, left) or P*Bs* (6 μg, right) and SecY (3.8 μg) were reconstituted into liposomes with (●) or without (○) SecG (1.8 μg), as described under "EXPERIMENTAL PROCEDURES." The translocation of [^{35}S]proOmpA D26 into the reconstituted proteoliposomes was then assayed at 37°C in the presence of SecA (60 μg/ml) and ATP (2 mM).

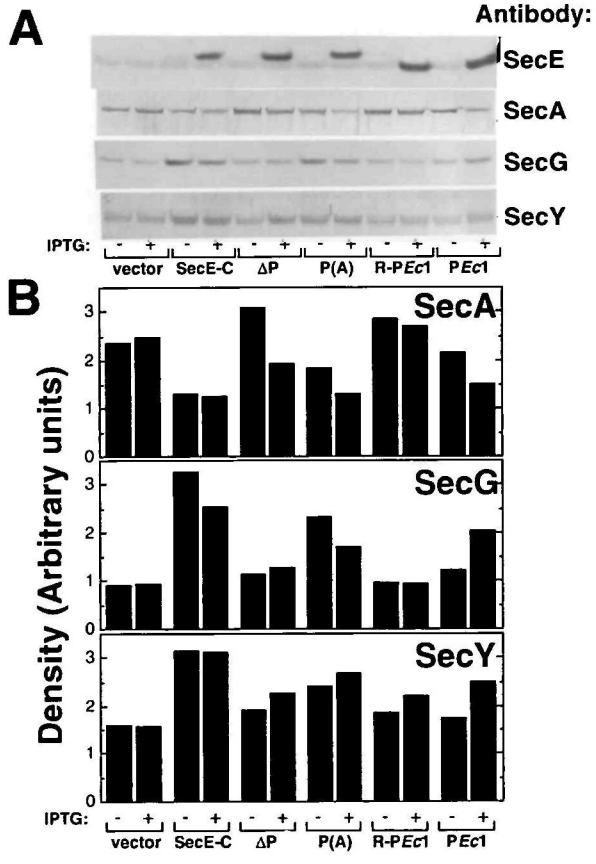

Fig. 5. **Levels of SecA, SecG, SecY, and SecE-C derivatives in *secEcsE501*.** PR520 (*secEcsE501*) cells harboring a plasmid encoding one of the following SecE-C derivatives were used: vector, pUSI2; SecE-C, pE1; ⊿P, pJVK55; P(A), pJVK84; R-P*Ec*1, pJVK44; P*Ec*1, pJVK48. Expression of these derivatives was induced with or without 1.5 mM IPTG at 20°C for 1.5 h. A: Levels of SecE-C derivatives, SecG, SecY, and SecA in whole cells (8 μg protein) were determined by SDS-PAGE and immunoblotting with the specified antibodies. B: Densitometric scanning of the band materials shown in A was carried out with a Shimadzu CS-930 chromatoscanner.

previously observed that the level of SecG in *secEcsE501* also decreased at 20°C (*9*). Taken together, these results suggest that the level of SecE determines those of SecY and SecG. On the other hand, the depletion of SecG had no effect on the levels of the other two factors (*10*). To determine whether or not the increases in the levels of SecY and SecG in the *secEcsE501* mutant depend on the SecE function, we expressed functional and non-functional SecE-C derivatives having an alteration in either the cytoplasmic region or the transmembrane region in *secEcsE501*, and then examined the levels of other Sec factors (Fig. 5A). The amount of each Sec factor was densitometrically determined (Fig. 5B). It should be noted that the level of SecE in *secEcsE501* was not affected by the expression of any SecE-C derivatives and remained low (data not shown). SecE-C and P(A), both of which are functional, affected the levels of other Sec factors even in the absence of IPTG owing to a leaky expression. Since the SecA expression is derepressed by a secretion defect (*2, 40*), the level of SecA in *secEcsE501* was higher in the absence than the presence of a functional SecE-C derivative. When a functional SecE-C derivative was expressed, the level of SecG appreciably increased. Excess expression of SecE-C and P(A) caused a slight decrease in the SecG level, however. Although the effect was less significant, the SecE function was also important for increasing the SecY level. Taken together, these results indicate that the levels of SecY and SecG in the membrane are determined by the functional level of SecE. FtsH, a multifunctional protease, has been shown to participate in the degradation of SecY, which is not associated with SecE (*41*). It is not known, however, how the SecG level is determined by SecE.

Although SecE is an essential component of the translocation machinery, its exact role remains to be clarified. The results presented in this paper may indicate that SecE plays only a limited, but essential, role in protein translocation or the formation of the translocation machinery. In addition to the four residues which we examined here, the cytoplasmic region contains some other conserved residues. Further systematic modifications, such as deletion, substitution and combination of them, are required to determine the minimum requirement of this region.

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