JOURNAL OF BACTERIOLOGY, Feb. 2003, p. 948–956 Vol. 185, No. 3
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.3.948–956.2003

A SecE Mutation That Modulates SecY-SecE Translocase Assembly, Identified as a Specific Suppressor of SecY Defects

Hiroyuki Mori, Yoshinori Akiyama, and Koreaki Ito*

Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

Received 30 September 2002/Accepted 4 November 2002

The SecY39(Cs) (cold-sensitive) alteration of Arg357 results in a defect of translocation initiation. As a means to dissect the Sec translocation machinery, we isolated mutations that act as suppressors of the *secY39* defect. A specific *secE* mutation, designated *secE105*, was thus isolated. This mutation proved to be identical with the *prlG2* mutation and to suppress a number of cold-sensitive *secY* mutations. However, other *prlG* mutations did not effectively suppress the *secY* defects. Evidence indicates that the Ser105-to-Pro alteration in the C-terminal transmembrane segment of SecE weakens SecY-SecE association. In vitro analyses showed that the SecE(S105P) alteration preferentially stimulates the initial phase of translocation. It is suggested that the S105P alteration affects the SecYEG channel such that it is more prone to open and to accept the translocation initiation domain of a preprotein molecule.

A significant fraction of *Escherichia coli* proteins are transported across the cytoplasmic membrane for their cell envelope localization. The major constituents of the Sec protein translocation system (translocase) include the SecA translocation ATPase and integral membrane components SecY, SecE, and SecG (16, 23). SecY is a central component of protein translocase, having 10 transmembrane (TM1 to TM10), 6 cytoplasmic (C1 to C6), and 5 periplasmic (P1 to P5) regions (3). Genetic and biochemical studies using cold-sensitive *secY* mutants (35) revealed that the carboxyl-terminal C5 and C6 regions of SecY are important for its function of activating the SecA translocation ATPase (35, 37). The *secY205* mutation affecting the C6 domain (Tyr429Asp) has a defect in the ATP- and pro-OmpA-dependent SecA insertion reaction in vitro, although futile insertion of SecA in the presence of an ATP analog [adenosine 5′-(β,γ-imido) triphosphate] is less impaired (18). Based on these results, we proposed that the *secY205* mutation changes the conformation of the SecYEG channel toward a more closed state. A number of suppressor *secA* mutations against the *secY205* defect were isolated (17, 18). Whereas some of them suppressed the defect allele-specifically, many others alleviated not only the original *secY205* defect but also other mutational *secY* defects, including *secY39* (see below). We called the latter class of suppressor variants "superactive" SecA, in which the intrinsic ATPase activities are indeed upregulated (17, 22, 24). Our mutation analysis also suggested that Arg357 in the SecY domain C5 is a functionally important residue (21). The superactive class of SecA variants allowed us to characterize the translocation defect caused by a strongly defective mutation at this residue. Characterization of the cold-sensitive mutation *secY39* (Arg357His) suggested that this mutation impairs the ability of translocase to utilize SecA effectively for initiation of translocation (22). Thus, this evolutionarily conserved Arg residue appears to have a key role in the activation of SecA.

* Corresponding author. Mailing address: Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan. Phone: 81-75-751-4015. Fax: 81-75-771-5699. E-mail: kito@virus.kyoto-u.ac.jp.

For translocation across the *E. coli* cytoplasmic membrane to occur, a preprotein must be targeted to the SecA/SecYEG translocase on the membrane. Then the translocation initiation domain that comprises the signal peptide and the early mature sequence of the preprotein will be inserted into the membrane. This is followed by signal peptide cleavage and continued forward movement of the mature region of the polypeptide. The initiation reaction that was mediated by the SecY39 form of translocase became measurable by use of a superactive SecA, but it still required the proton motive force (PMF) almost absolutely. We proposed that the PMF might facilitate the initiation reaction by inducing a conformational change of the channel. Such an effect might be similar to the *prlA* mutation-induced relaxation of the SecYEG channel, which also results in a decreased dependence on the PMF as well as in an increased affinity for SecA (8, 27, 38).

This paper describes additional suppression studies of the *secY39* mutation. A SecE alteration has thus been identified that alleviates the initiation defect of the SecY39 alteration. The SecE alteration also suppressed a number of other *secY* mutations.

MATERIALS AND METHODS

Media and transduction. Minimal medium M9 was as described previously(33). L-medium contained 10 g of Bacto Tryptone, 5 g of yeast extracts, and 5 g of NaCl per liter. P-medium contained 20 g of Polypeptone, 5 g of NaCl, and 5.6 mmol of NaOH per liter. It was supplemented with 25 μg of tetracycline/ml as required for selection. P1 transduction was carried out by standard procedures (20), and unselected markers were scored as described previously (18).

***E. coli* strains.** *E. coli* strains used in this study are listed in Table 1. A Tet^s derivative of the *secY39* mutant, GN31 (AD202 *secY39*), was the starting strain for general isolation of suppressor mutations on the chromosome. Tet^s derivatives GN15 (*secY205*), GN32 (*secY125*), and GN33 (*secY104*) (18) as well as GN31 (*secY39*) were used for examination of the allele specificity of the suppressor. Strains TW156 (*secY*$^+$), GN4 (*secY39*), GN5 (*secY205*), GN9 (*secY104*), HM808 (*secY39 secE105*), HM809 (*secY205 secE105*), and HM810 (*secY104 secE105*) were derived from strain AD202 (1) and carried the Δ(*uncB-uncC*) marker (15) in addition to the *secY* and *secE* mutations indicated. These strains were used for preparation of inverted membrane vesicles (IMVs). Strains HM808, HM809, and HM810 were constructed by four rounds of P1 transduction experiments.

TABLE 1. Bacterial strains

Strain	Relevant genotype	Reference or source
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*	33
AD202	MC4100 *ompT*::*kan*	2
EM100	MC4100 *secY24 rpsE*/F′ *lacI*^{q} *lacPL8 lacZ*$^+$*Y*$^+$*A*$^+$ *pro*$^+$	19
EM146	AD202/F′ *lacI*^{q} *lacPL8 lacZ*$^+$*Y*$^+$*A*$^+$ *pro*$^+$	Laboratory stock
GN04	AD202 *secY39 zhd33*::Tn*10* Δ(*uncBC*)	Laboratory stock
GN05	AD202 *secY205 zhd33*::Tn*10* Δ(*uncBC*)	18
GN09	AD202 *secY104 rpsE zhd33*::Tn*10* Δ(*uncBC*)	Laboratory stock
GN15	AD202 *secY205* Tet^s	18
GN17	AD202 *secY205 leu82*::Tn*10*	18
GN31	AD202 *secY39* Tet^s	17
GN32	AD202 *secY125 rpsE* Tet^s	17
GN33	AD202 *secY104 rpsE* Tet^s	17
HM541	AD202 *secY39 secE105 yijP*::Tn*10*	This study
HM545	AD202 *secY39 secE*$^+$ *yijP*::Tn*10*	This study
HM546	AD202 *secY205 secE105 yijP*::Tn*10*	This study
HM547	AD202 *secY205 yijP*::Tn*10*	This study
HM548	AD202 *secY104 rpsE secE105 yijP*::Tn*10*	This study
HM549	AD202 *secY104 rpsE yijP*::Tn*10*	This study
HM550	AD202 *secY125 rpsE secE105 yijP*::Tn*10*	This study
HM551	AD202 *secY125 rpsE yijP*::Tn*10*	This study
HM562	AD202 *secE*$^+$ *yijP*::Tn*10*	This study
HM564	AD202 *secE105 yijP*::Tn*10*	This study
HM695	EM100 *secY24 secE*$^+$ *yijp*::Tn*10*/pHMC5A	This study
HM696	EM100 *secY24 secE105 yijp*::Tn*10*/pHMC5A	This study
HM808	AD202 *secY39 zhd33*::Tn*10 secE105* Δ(*uncBC*)	This study
HM809	AD202 *secY205 zhd33*::Tn*10 secE105* Δ(*uncBC*)	This study
HM810	AD202 *secY104 rpsE zhd33*::Tn*10 secE105* Δ(*uncBC*)	This study
HM811	AD202 *secY*$^+$ *zhd33*::Tn*10 secE105* Δ(*uncBC*)	This study
HM834	EM146 *secE*$^+$ *yijp*::Tn*10*	This study
HM835	EM146 *secE105 yijP*::Tn*10*	This study
TW156	AD202 *zhd33*::Tn*10* Δ(*uncBC*)	18

Plasmids. Plasmids used in this study are listed in Table 2. Plasmids pHM401 (PrlG1), pHM402 (PrlG2), and pHM403 (PrlG3), encoding PrlG derivatives, were constructed by the QuikChange method (Stratagene), based on pCM134 (SecE$^+$) and using appropriate mutagenic primers. pBR-based plasmids pHMC5A (SecY$^+$), pHMC5Δ12 [SecY Δ(346-357)], and pR357E [SecY(R357E)], encoding the SecY and SecY derivatives indicated (21), were used for examination of dominant-negative phenotypes. Plasmid pNA3, encoding SecY-His$_6$-Myc, was constructed as follows. Plasmid pHMC5A was subjected to site-directed mutagenesis for the stop codon region of *secY* (mutagenic primers, 5′-GGCTACGGCCGAGGATCCGTCGCCCGAGAAGT-3′ and its complementary strand [the *Bam*HI recognition sequence is underlined]) such that the stop codon was converted to that for Ser and the *Bam*HI site was introduced simultaneously. Two oligonucleotide linkers, encoding the Gly-Ser-His$_6$-Leu-Gln sequence and the Myc epitope sequence (Leu-Gln-Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Leu-Arg-Lys-Arg), were sequentially inserted between the introduced *Bam*HI site and multicloning sites of the plasmid to produce pNA3. pACYC-based plasmids pCM10 (SecY$^+$), pHM404 [SecY Δ(346-357)], and pHM405 (SecY R357E) were used for measurement of in vivo stability of the SecY derivatives.

Isolation of *secY39*-suppressing mutations. To isolate suppressor mutations on the chromosome, cells of GN31 (*secY39*) were UV irradiated (14 J/m^2 for 10 s) and divided into 10 portions, which were grown on L medium overnight and plated on L agar. Colonies that appeared after 3 days of incubation at 20°C (at an apparent frequency of about 10^{-5}) were pooled. Ten independent mutants were characterized by P1 transduction. Two of them had mutations cotransducible with *leu82*::Tn*10*, probably in *secA*. Seven of them had suppressor mutations linked with *secY39* itself, probably within *secY*. We isolated a Tn*10* insertion that was cotransducible with the remaining suppressor mutation after a random Tn*10*

TABLE 2. Plasmids

Plasmid	Cloned gene	Promoter	Replicon	Reference
pHM401	*prlG1*	*lac*	pBR322	This study
pHM402	*secE105 (prlG2)*	*lac*	pBR322	This study
pHM403	*prlG3*	*lac*	pBR322	This study
pHM404	*secY* Δ(*346–357*)	*lac*	pACYC184	This study
pHM405	*secY(R357E)*	*lac*	pACYC184	This study
pHMC5A	*secY*$^+$	*lac*	pBR322	21
pHMC5Δ12	*secY* Δ(*346–357*)	*lac*	pBR322	This study
pNA3	*secY*-His$_6$-*myc*	*lac*	pBR322	This study
pR357E	*secY (R357E)*	*lac*	pBR322	21
pCM10	*secY*$^+$	*lac*	pACYC184	19
pCM134	*secE*$^+$	*lac*	pBR322	E. Matsuo, unpublished data
pSTD343	*lacI*^{q}	*lac*	pACYC184	Y. Akiyama, unpublished data
pTWV228	Vector		pBR322	35
pSTV28	Vector		pACYC184	31

transposition followed by P1 transduction (4, 14). The transposon insertion point was determined by the direct inverse PCR method (25) to be within the *yijP* open reading frame. This chromosomal location (89.5 min) is close to that of *secE* (90.08 min).

Pulldown assay with Ni-NTA resin. Plasmid pNA3 has full complementation activity against the *secY39* defect (data not shown). The plasmid was introduced into cells of HM562 (*secE*$^+$)/pSTD343 and HM564 (*secE105*)/pSTD343. Expression of SecY-His_6-Myc was induced for 1 h by addition of 1 mM isopropyl-β-D-thiogalactoside (IPTG) and 5 mM cyclic AMP at early-log phase. Total membrane fractions were prepared (40), and portions containing 100 μg of proteins were solubilized with 2% (wt/vol) *n*-dodecyl-β-D-maltopyranoside (DM) by incubation on ice for 30 min. Insoluble materials were removed by ultracentrifugation. After incubation of the supernatant for 30 min at various temperatures, samples were applied to a Ni-nitrilotriacetic acid (NTA) spin column (Qiagen) equilibrated with 50 mM Tris-HCl (pH 7.5) containing 0.3 M NaCl, 20% glycerol, 10 mM imidazole, and 0.03% DM. The column was washed three times with 300 μl of the above buffer, and the tagged SecY was eluted with 200 μl of elution buffer containing 1 M imidazole. All the samples were precipitated with 5% trichloroacetic acid (TCA). The precipitates were washed with acetone and dissolved in sodium dodecyl sulfate (SDS) sample buffer. Then the samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (11) and immunoblotting with anti-SecY (31), anti-SecE (which was raised against a synthetic peptide with a sequence corresponding to residues 1 to 16 of SecE), anti-SecG (26), and anti-Myc (purchased from Santa Cruz Biotechnology) antibodies.

In vivo protein export and in vitro translocation assays. In vivo protein export and in vitro translocation assays were carried out according to procedures reported previously (21).

RESULTS

A SecE alteration that suppresses the SecY39 defect. The *secY39* mutation (5) alters Arg357, an essential SecY residue (21), to His, and retards protein export and cell growth at 20°C. We isolated its suppressor mutations on the chromosome, as described in Materials and Methods. Among these, we identified a mutation that was P1 cotransducible, at a frequency of about 40%, with a Tn*10* insertion within the *yijP* open reading frame. This location was consistent with the suppressor mutation having occurred in *secE* (20). Sequencing of the *secE* region indeed revealed a base substitution, T313 to C within *secE*, that would result in a Ser105-to-Pro amino acid change in the SecE protein. This mutation was designated *secE105*, and its product was designated SecE(S105P). The amino acid alteration is identical to that caused by the *prlG2* mutation, isolated by others as a suppressor mutation against mutational defects of the signal sequence of LamB (29, 34).

The following results suggested that the suppression of the *secY39* mutation was indeed due to the *secE105* mutation. First, upon sequencing of the P1 transductants, suppression and the base change coincided, although the number of sequenced transductants was limited. Second, we carried out independent isolation of suppressor mutations in the *secE* region by localized mutagenesis using a combination of the *yijP*::Tn*10* marker and P1 transduction. We obtained a mutant that carried the same nucleotide alteration as the *secE105* mutation. No other *secE* mutation has been obtained so far.

Pulse-chase experiments showed that the suppressed *secY39-secE105* cells had recovered significant protein export ability compared with the nonsuppressed *secY39* mutant. This was demonstrated for export of maltose-binding protein (MBP) and OmpA both at 37°C and at 20°C (Fig. 1A; compare lanes 1 to 4 with lanes 5 to 8).

The identity of the SecE(S105P) and the PrlG2 amino acid alteration raised the question of whether all *prlG* mutations can suppress *secY39*. To examine this point, we expressed SecE$^+$ as well as the PrlG1 (Leu108Arg), PrlG2 (Ser105Pro), and PrlG3 (Ser120Phe) forms of SecE from plasmids in the *secY39* mutant cells. Previous studies had showed that the *prlG* phenotypes can be expressed dominantly (9). It was found that among the three mutant SecE proteins, only SecE(S105P) (PrlG2) alleviated the growth defect at 20°C (Fig. 1B, lane 3). The protein export defect was also most effectively suppressed by this mutant form of SecE (Fig. 1C, lower panel, lane 3) at 20°C. Interestingly, expression of the PrlG1 form of SecE in the *secY39* background appeared to slightly exacerbate the protein export defect, as revealed by the results at 37°C (Fig. 1C, upper panel, lane 2). Thus, the S105P alteration is specific among the PrlG class of SecE alterations in the ability to effectively suppress the *secY39* mutation. Indeed, we repeatedly obtained the *secE105* alteration on a plasmid after selection for a dominant suppressor of *secY39* (H. Mori and K. Ito, unpublished data).

SecE(S105P) compensates for a number of SecY mutational defects. To examine the specificity of suppression by the *secE105* mutation, it was introduced into several *secY* cold-sensitive mutants (35). P1 transduction experiments showed that mutants *secY205*, *secY104*, and *secY125* were all suppressed with respect to the cold sensitivity in growth (data not shown) and protein export defects (Fig. 2A). Thus, the suppression is not *secY* allele specific. Similar wide-range suppression was observed with the superactive class of *secA* mutations (17). The *secE105* single mutant strain itself was normal with respect to cell growth and to export of MBP and OmpA (data not shown).

When a mutant form of SecY with certain amino acid alterations in cytoplasmic domain 5 is expressed from a plasmid, it interferes with protein export in the presence of chromosomally encoded SecY$^+$ (21, 32). These SecY molecules are thought to be inactive but still capable of binding SecE and thus sequestering it. Cytoplasmic domain 4 is important for the SecY-SecE interaction and, hence, for this dominant interference (6, 19, 32). We now found that the dominant-negative forms of SecY, such as SecY(R357E) and SecY Δ(346-357) (a deletion of a Leu346-to-Arg357 segment), no longer interfered with export of MBP and OmpA when the host strain had the chromosomal *secE105* mutation (Fig. 2B). Similar results were obtained with other dominant-negative point mutations of *secY* as well as with the SecY-(P5)-PhoA fusion protein lacking a C-terminal (TM10 to C6) region of SecY (data not shown). These results suggest that the SecE(S105P) alteration either restores the functionality of the mutant SecY protein or preferentially stabilizes the wild-type SecY protein.

SecE(S105P) exacerbates the SecY24 defect with weakened SecY-SecE interaction. A temperature-sensitive *secY* mutation, *secY24*, alters a residue in the C4 region (30) and weakens SecY-SecE interaction (6, 19). As a result, the altered SecY protein is degraded by the FtsH protease at 42°C (13). Attempts to construct a *secY24-secE105* double mutant by P1 transduction were successful only when the recipient *secY24* mutant carried a plasmid with *secY*$^+$ cloned under the control of the *lac* promoter and when the entire transduction experiments were carried out in the presence of IPTG. The resulting transductants that received *secE105* exhibited IPTG-dependent growth (Fig. 3A), indicating that the *secY24* and *secE105* mutations result in a synthetic lethal phenotype.

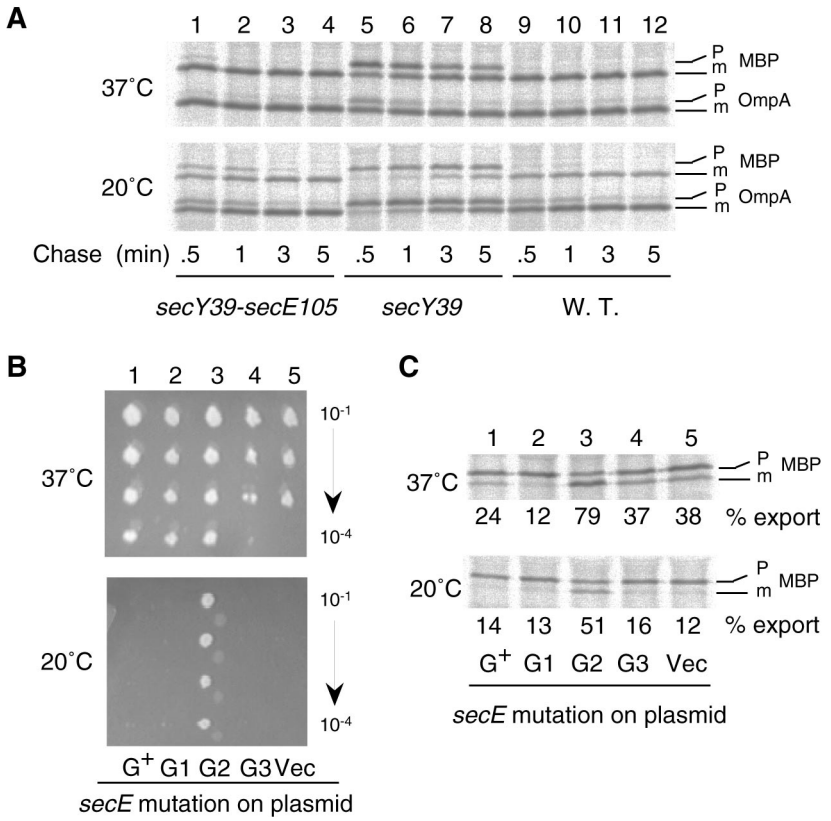

FIG. 1. Effects of the *secE105* mutation on *secY39* defects. (A) Effects on protein export. Strains HM541 (*secY39 secE105*) (lanes 1 to 4), HM545 (*secY39 secE*$^+$) (lanes 5 to 8), and AD202 (*secY*$^+$ *secE*$^+$) (lanes 9 to 12) were grown at 37°C in M9-glycerol-amino acids medium supplemented with maltose (0.4%) until early-log phase (upper panel). A portion of the cultures was then shifted to 20°C for 30 min (lower panel). Cells were pulse-labeled with [^{35}S]methionine for 0.5 min (at 37°C) or 1 min (at 20°C) and chased with unlabeled methionine for the indicated periods. At each time point, a portion of culture was directly treated with TCA (final concentration, 5%), and protein precipitates were solubilized and processed for immunoprecipitation of MBP and OmpA. Their precursor (p) and mature (m) forms were visualized by phosphor image analyzer after SDS-PAGE. (B) Effects of *prlG* expression on growth of the *secY39* mutant. Strain GN31 (*secY39*) was transformed with either pCM134 (*secE*$^+$), pHM401 (*prlG1*), pHM402 (*prlG2*), pHM403 (*prlG3*), or pTWV228 (vector). The transformant cultures, grown at 37°C in L-ampicillin (50 μg/ml), were subjected to 10-fold serial dilutions (from top to bottom) and spotted onto L-ampicillin agar plates, which were then incubated at 37°C for 16 h or at 20°C for 60 h, as indicated. (C) Effects of *prlG* expression on protein export. The transformant cells shown above were grown at 37 or 20°C, subjected to pulse-labeling, and processed for immunoprecipitation of MBP, as described in the legend to panel A, but without a chase. The percentage of the mature form of MBP is shown below each lane.

Effects of the SecE(S105P) alteration on SecY-SecE interaction. We found that the SecY24 mutant protein is destabilized in the above double-mutant cells. When IPTG was removed from a culture of the double-mutant strain, growth was slowed down after about 3 h (data not shown). Presumably, at this time the wild-type SecY protein had been sufficiently diluted. We found that after about 3 h of growth in the absence of IPTG, the abundance of the SecY24 protein in cells decreased strikingly (Fig. 3B, lanes 2 and 3), in contrast to background proteins that fortuitously served as internal controls (Fig. 3B). Control cells with the wild-type *secE* gene contained only marginally decreased amounts of SecY24 mutant protein after IPTG removal (Fig. 3B, lanes 5 and 6). It should be noted that singly overexpressed SecY molecules accumulate only to a limited extent, because those molecules that fail to associate with SecE will be degraded rapidly (13; also see below). The results obtained indicate that the SecY24 mutant protein is unstable in combination with the S105P form of SecE. The synthetic lethal and SecY degradation phenotypes of the *secY24-secE105* double mutant suggest a possibility that both of these mutations impair the SecY-SecE interaction such that their combination leads to the lethal inability to form a translocation channel. We confirmed, by Western blot experiments, that the SecE(S105P) mutant protein accumulated normally in the cell (data not shown). This excludes the possibility that the lethal combination with SecY24 was due to instability of the SecE(S105P) protein itself.

To examine whether SecE(S105P) can interact with SecY normally, pulldown experiments were performed using a plasmid expressing SecY-His$_6$-Myc. Thus, membrane fractions were prepared from either the *secE*$^+$ or the *secE105* strain carrying a *secY*-His$_6$-*myc* plasmid. Membranes were solubilized with 2% DM and preincubated at various temperatures for 30 min. Samples were then subjected to Ni^{2+}-NTA affinity isolation. The wild-type SecE-SecY complex did not dissociate at temperatures up to 37°C in this detergent solution (Fig. 4, lanes 1 to 3, for preincubation up to 26°C). Thus, this detergent allowed more-stable SecY-SecE association than Triton X-100,

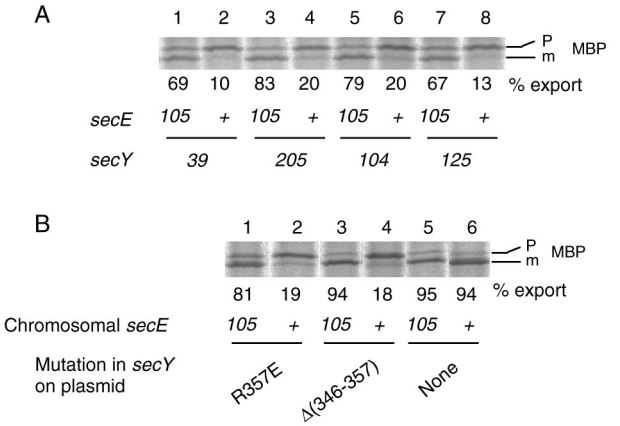

FIG. 2. Specificity of suppression by *secE105*. (A) Effects on chromosomal *secY* mutations. Cold-sensitive *secY* mutants, as indicated, with or without additional *secE105* mutation, were grown first at 37°C until early-log phase and then at 20°C for 30 min. Cells were pulse-labeled with [^{35}S]methionine for 1 min and immediately processed for immunoprecipitation of MBP. Labeled MBP molecules were visualized. p, precursor forms; m, mature forms. (B) Effects on dominant-negative *secY* mutations on plasmids. Strains HM562 (*secE*$^+$) and HM564 (*secE105*), each carrying plasmid pSTD343 (*lacI*^{q}), were further transformed with a compatible plasmid encoding wild-type or mutant SecY. The mutant forms, SecY(R357E) and SecY Δ(346-357), are known to be dominant negative (21). Cells were grown at 37°C in the presence of 1 mM IPTG, 5 mM cyclic AMP, 50 μg of ampicillin/ml, and 20 μg of chloramphenicol/ml and were pulse-labeled with [^{35}S]methionine for 0.5 min.

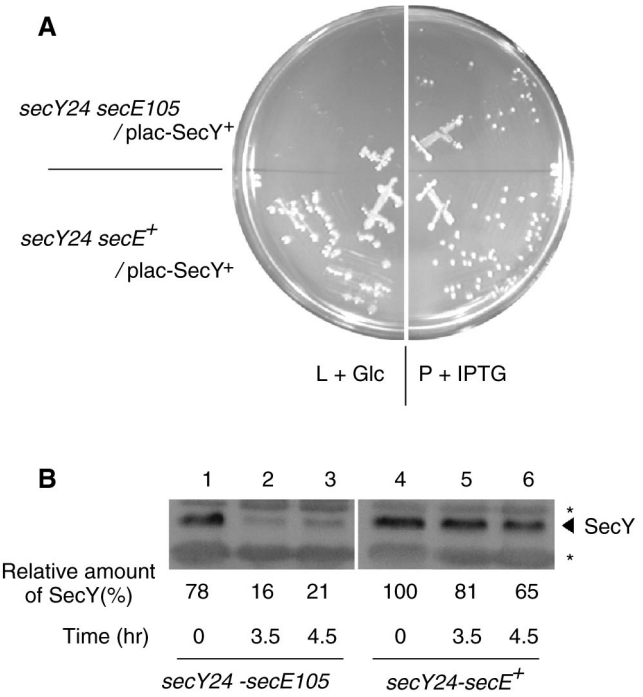

FIG. 3. Mutations *secE105* and *secY24* are synthetically lethal, with destabilization of the SecY24 protein. (A) The *secE105* mutation was introduced by P1 transduction into strain EM100 (*secY24* F′ *lacI*^{q}) carrying pHMC5A (*secY*$^+$ under the control of the *lac* promoter) in the presence of IPTG to produce HM696. HM695 was a control strain (*secE*$^+$ *secY24*/pHMC5A). Cell growth on L agar supplemented with 0.4% glucose (L + Glc) and P agar supplemented with 1 mM IPTG (P + IPTG) was recorded after incubation at 30°C for 18 h. (B) Strains HM696 (*secY24 secE105*) (see above) and HM695 (its *secE*$^+$ derivative) were grown at 30°C in P medium supplemented with 1 mM IPTG and 50 μg of ampicillin/ml to early-log phase. Cells were then collected, washed, and inoculated into L medium containing 0.4% glucose, followed by further incubation at 30°C. Samples were withdrawn at the indicated time points and subjected to anti-SecY immunoblotting, with sample sizes adjusted to those corresponding to a fixed approximate cell number. Arrowhead indicates SecY. Asterisks show background cross-reacting bands. The intensity of each SecY band, relative to that in lane 4, is shown below each lane.

which was used in our previous analysis (10). SecE(S105P) was recovered in the imidazole eluate fraction less efficiently than SecE$^+$ (Fig. 4; compare lanes 1 and 4); a substantial fraction of the mutant protein was in the flowthrough fraction (data not shown). The SecY-His$_6$-Myc-SecE(S105P) complex was preserved upon preincubation at 16°C (Fig. 4, lane 5) but not at 26°C (lane 6) or higher. These results demonstrate that the *secE105* mutation weakens SecY-SecE interaction under detergent-solubilized conditions.

To obtain further information on the in vivo ability of the SecE(S105P) mutant protein to interact with different SecY variants, its ability to stabilize overexpressed SecY molecules was examined by pulse-chase experiments. We did these in vivo experiments because the defect observed after the detergent solubilization might not have reflected exactly the in vivo state, in which these proteins were integrated into the lipid bilayer (6, 9). Whereas overexpressed SecY was degraded with a half-life of about 2 min (36) (data not shown), simultaneous overproduction of SecE as well as that of SecE(S105P) effectively stabilized it (Fig. 5, lanes 1 to 5). The wild-type and mutant forms of SecE were overproduced and accumulated equally (data not shown). Although the above result is not surprising, since the *secE105* mutant cells are fully viable and the interaction defect, if any, seems mild, it does not explain the suppression of the dominant-negative effects of the *secY* mutations. Two possibilities are conceivable. First, SecE(S105P) enables the mutant SecY protein to function. Second, it selectively interacts with wild-type SecY molecules and less efficiently with the inactive SecY mutant molecules. Pulse-chase experiments showed that SecY(R357E) was stabilized by both SecE$^+$ and SecE(S105P) (Fig. 5, lanes 11 to 15). In contrast, SecY Δ(346-357) was not significantly stabilized by SecE(S105P) (Fig. 5, upper panel, lanes 6 to 10) (data not shown for the vector control), whereas it was stabilized normally by SecE$^+$ (Fig. 5, lower panel, lanes 6 to 10). These results indicate that SecE(S105P) has a significant interaction defect in vivo with SecY24 and SecY Δ(346-357). The C5 region affected by the SecY Δ(346-357) mutation may also participate in the SecY-SecE interaction. Indeed, the C5 region of SecY is in close proximity to the C2 region of SecE (Y. Satoh, H. Mori, and K. Ito, unpublished data).

In vitro effects of SecE(S105P) on protein translocation. Mutational effects were characterized in in vitro translocation assays. IMVs were prepared from *secY*$^+$, *secY39*, *secY205*, and *secY104* cells as well as from these mutants with an additional *secE105* suppressor mutation. Their pro-OmpA-translocating activities were assayed in the presence of SecA and ATP. Reactions were allowed either at 37°C or at 20°C in the pres-

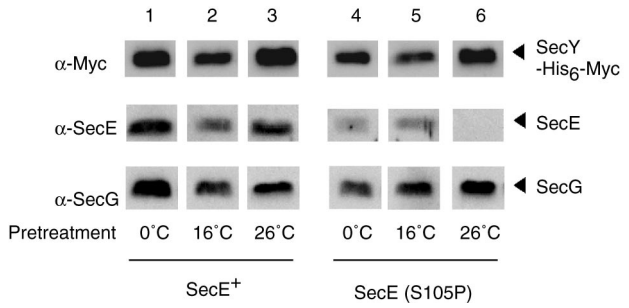

FIG. 4. Stability of the SecY-SecE association in DM. Two compatible plasmids, pSTD343 (*lacI*^{q}) and pNA3 (*secY*-His$_6$-*myc*), were introduced into strains HM562 (*secE*$^+$) and HM564 (*secE105*). Membrane fractions were prepared from *lac*-induced cultures of the plasmid-bearing cells, and portions containing 100 μg of protein were solubilized with 2% DM. Supernatants after centrifugation (at 100,000 × *g* for 30 min at 4°C) were incubated at the indicated temperature for 30 min and then applied to Ni-NTA spin columns (Qiagen), which were washed with a buffer containing 0.03% DM and eluted with 1 M imidazole in the same buffer. Eluates were subjected to SDS-PAGE and immunoblotting with the antibodies shown on the left.

ence or absence of the PMF imposed across the membrane (see Materials and Methods). We noticed that IMVs prepared from the *secY205* or *secY104* mutant were partially inactivated during storage at −80°C. Therefore, the results presented in Fig. 6 were obtained with freshly prepared IMVs. Although IMVs from *secY205* or *secY104* mutant cells showed almost normal translocation activities at 37°C in the presence of the PMF (Fig. 6A, lanes 7 and 11), their activities decreased markedly in the absence of the PMF (lanes 8 and 12). This reduction was especially evident at 20°C. In contrast, IMVs from the suppressed versions of these mutants were fully active (Fig. 6A; see the full-length OmpA band for lanes 6 and 10) and only slightly affected by PMF.

The *secY39* IMV showed greatly reduced translocation activity at 37°C even in the presence of the PMF and was virtually inactive in the absence of the PMF or at 20°C (Fig. 6A, lanes 3 and 4). The SecE(S105P) alteration had only a slight (Fig. 6A, lane 2) or negligible stimulatory effect on translocation in

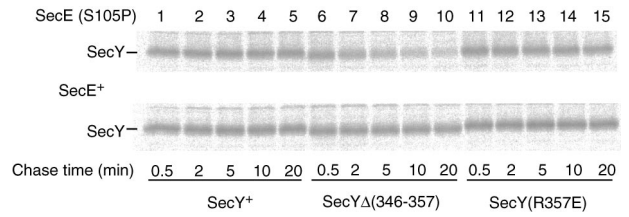

FIG. 5. SecY-stabilizing ability of SecE(S105P). Strain HM834 (*secE*$^+$) or HM835 (*secE105*) was transformed with two compatible plasmids, one overexpressing SecY or its derivative and another overexpressing SecE$^+$ (lower panel) or SecE(S105P) (upper panel), respectively. The SecY plasmids used were pCM10 (SecY$^+$) (lanes 1 to 5), pHM404 [SecY Δ(346-357)] (lanes 6 to 10), and pHM405 [SecY(R357E)] (lanes 11 to 15). Cells were grown in the presence of 1 mM IPTG and 5 mM cyclic AMP at 37°C and were pulse-labeled with [^{35}S]methionine for 30 s, followed by a chase with unlabeled methionine for the indicated periods. The labeled SecY proteins were immunoprecipitated and visualized by phosphor image analyzer after SDS-PAGE.

the presence of the SecY39 alteration. The lack of significant translocation activities for the SecY39-SecE(S105P) IMV was unexpected from the results obtained in vivo (Fig. 1A).

We characterized the effects of the SecE(S105P) alteration itself on the in vitro pro-OmpA translocation reaction (Fig. 6B). At 20°C and in the presence of the PMF, the SecE(S105P) IMV was about 50% as active as the wild-type IMV (Fig. 6B, lanes 1 to 5 versus lanes 11 to 15, and Fig. 6C). We noticed that the mutant reaction led to accumulation of higher proportions of lower-molecular-mass translocated products than the wild-type reaction (Fig. 6B and D). These products probably represented incompletely translocated molecules. At this low temperature, the wild-type reaction exhibited a strong dependence on the PMF. This was true both for the full-length translocation and for the incomplete translocation (Fig. 6B; compare lanes 11 to 15 with lanes 16 to 20). Interestingly, in the absence of the PMF, the SecE(S105P) IMV was more active, both for the full-length translocation and for the incomplete translocation, than the wild-type IMV (Fig. 6B [compare lanes 6 to 10 and 16 to 20], C, and D). Thus, the PMF dependence in translocation was somehow alleviated in the SecE105 mutant IMV. This SecE alteration might have been primarily responsible for the decreased PMF dependence observed with the IMVs from the suppressed *secY205* and *secY104* mutants (Fig. 6A).

The fact that the SecE(S105P) alteration compromised the translocation completion function of translocase might explain its inability to restore the SecY39 mutational defects in vitro. When the reaction with the SecY39-SecE(S105P) IMV was compared with the reaction with the SecY$^+$ IMV, the former showed a strikingly enhanced appearance of lower-molecular-mass products that had been protected from proteinase K digestion (Fig. 7A, lanes 6 to 10). The results of quantitation of each of these bands and the full-length translocation product (Fig. 7C) are consistent with the notion that the incompletely translocated molecules represent translocation intermediates; at least I_{16} for the reaction with the double-mutant IMV and I_{26} for the reaction with wild-type IMV exhibited a transient increase that was followed by a decrease, whereas the full-length product continued to increase during these periods.

These results suggest that the SecY39-SecE(S105P) translocase can function in the early phases of the translocation reaction but is less active in overcoming the energy barriers that pro-OmpA translocation encounters later, at specific regions along its polypeptide chain. In accordance with this interpretation, the SecY39-SecE(S105P) IMV showed only a moderate delay in the processing of the signal peptide of pro-OmpA, an early event in the translocation processes (Fig. 7B [compare lanes 2 to 6 and 7 to 11] and C). The SecY39 single-mutant IMV was inactive in the processing reaction (Fig. 7A, lane 1). The kinetics of the appearance of different pro-OmpA species are consistent with the notion that signal sequence processing was the earliest event (Fig. 7C).

A superactive form of SecA (SecA329) can drive efficient pro-OmpA translocation into the SecY39 IMV (data not shown), and this enabled the following experiments. To examine whether I_{16} generated in the SecY39-SecE(S105P) IMV-mediated translocation reaction indeed represented an intermediate state of translocation, the pro-OmpA translocation reaction was allowed for 5 min to accumulate I_{16}, and then

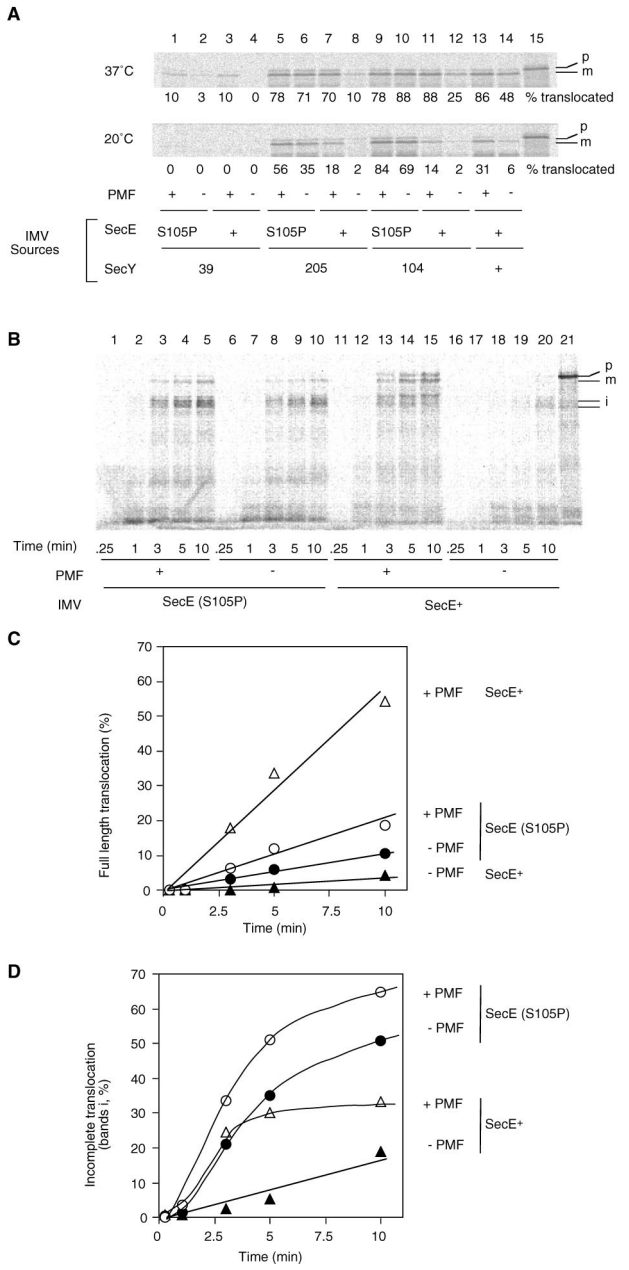

FIG. 6. In vitro characterization of SecE(S105P) effects on pro-OmpA translocation. (A) Effects on different SecY mutational defects. IMVs were prepared from strains HM808 (*secY39 secE105*) (lanes 1 and 2), GN04 (*secY39*) (lanes 3 and 4), HM809 (*secY205 secE105*) (lanes 5 and 6), GN05 (*secY205*) (lanes 7 and 8), HM810 (*secY104 secE105*) (lanes 9 and 10), GN09 (*secY104*) (lanes 11 and 12), and TW156 ($secY^+$ $secE^+$) (lanes 13 and 14). They were subjected to an in vitro translocation assay using ^{35}S-labeled pro-OmpA and wild-type SecA. The PMF was generated (+) or dissipated (−) by addition of 5 mM succinate or 10 μM carbonycyanide-*m*-chlorophenyl hydrazone, respectively. Reactions were allowed to proceed for 10 min at 37°C (upper panel) or 20°C (lower panel). Samples were treated with proteinase K and analyzed by SDS-PAGE and phosphorimager exposure. p, precursor forms; m, mature forms. (B) pro-OmpA translocation reaction mediated by the SecE(S105P) single-mutant IMV. IMVs were prepared from strains HM811 (*secE105*) (lanes 1 to 10) and TW156 ($secE^+$) (lanes 11 to 20). They were subjected to a ^{35}S-labeled pro-OmpA translocation assay at 20°C in the presence (+) or absence (−) of the PMF for the indicated lengths of time. Shown are SDS-PAGE profiles after proteinase K treatment. i, fragments generated from incompletely translocated pro-OmpA. (C) Time courses of full-length translocation. Intensities of the full-length proOmpA product (mature and precursor forms) with a SecE(S105P) IMV (circles) or a Sec^+ IMV (triangles) are plotted against the reaction time. Open and solid symbols, results in the presence and absence of the PMF, respectively. (D) Time courses of generation of incompletely translocated products. Intensities of the incomplete translocation products (corresponding to the "i" bands in panel B) are plotted against the reaction time. Symbols are the same as in panel C.

SecA329 was added. As a control, wild-type SecA was added. Whereas addition of wild-type SecA did not significantly change the intensity of I_{16} (Fig. 7D, lanes 12 to 14; Fig. 7E, right graph), addition of SecA329 significantly reduced the I_{16} intensity, with a concomitant increase in the intensity of I_{26} as well as in that of the full-length translocation product (Fig. 7D, lanes 5 to 7; Fig. 7E, left graph). These results suggest that I_{16} was a translocation intermediate. The SecE(S105P) alteration effectively compensated for the SecY39 defect in the earlier steps but less effectively in the later steps.

DISCUSSION

The SecY39 alteration of the SecYEG translocase impairs its preprotein translocation functions, and this defect can be alleviated by a superactive form of SecA, but only in the presence of the PMF imposed across the membrane. Evidence suggested that the PMF is required at the initiation step of translocation. The enhanced activity of the SecA ATPase can overcome only the later steps of translocation, i.e., after insertion of the "translocation initiation domain" (22). The present results indicate that the initiation defect of the SecY39 translocase can also be alleviated by an alteration in the SecE subunit. The Ser105-to-Pro alteration by the *secE105* (*prlG2*) mutation within the third transmembrane segment of SecE is responsible for this suppression effect.

The Prl alterations of the Sec components are thought to somehow compromise the proofreading ability of the translocase, thereby making it accept wider ranges of preproteins (28). It has been reported also that some Prl mutations weaken SecY-SecE interaction (8), lower the PMF dependence of translocation (8, 27), and/or increase the SecA-binding affinity (38). All of these observations suggest that these alterations make the translocation channel more open. Our present results revealed that one specific alteration in SecE, Ser105Pro, can compensate for the initiation defect due to the SecY39 channel alteration. Among different *prlG* mutations examined, the ability to suppress *secY39* was observed most clearly for *prlG2* (*secE105*); neither the *prlG1* mutation nor the *prlG3* mutation effectively suppressed the *secY39* or the *secY205* mutation (data for effects on *secY205* not shown). In fact, the *prlG1* mutation exerted an opposite effect on the *secY39* mutant phenotype. Thus, suppression of signal sequence defects and suppression of the SecY defects have some distinct features. Repeated and selective isolation of the S105P alteration in SecE after chromosomal localized mutagenesis as well as after plasmid mutagenesis indicates that this alteration is specific in the ability to alleviate the *secY39* defect of SecY.

Our in vitro results suggest that the SecE(S105P) alteration

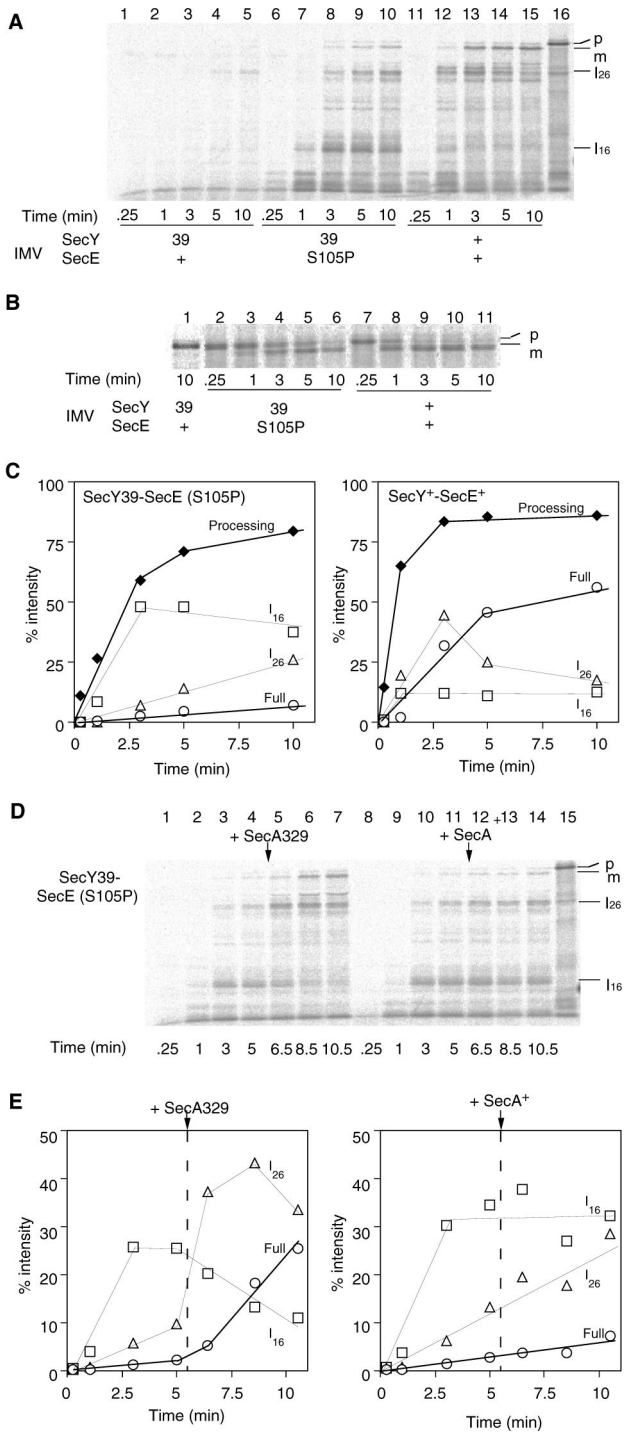

FIG. 7. SecE(S105P) preferentially compensates for the early translocation defect of the SecY39 alteration. (A) Translocation. IMVs prepared from strains GN04 (*secY39*) (lanes 1 to 5), HM808 (*secY39 secE105*) (lanes 6 to 10), and TW156 (*secY*$^+$) (lanes 11 to 15) were subjected to a ^{35}S-labeled pro-OmpA translocation reaction at 37°C with wild type SecA and in the absence of the PMF. At the indicated time points, samples were treated with proteinase K and analyzed by SDS-PAGE and phosphorimaging. Positions of precursor (p) and mature (m) forms, as well as those of two intermediate forms (I_{26} and I_{16}), are shown. (B) Processing. The reactions for which results are shown in panel A were terminated directly with 5% TCA. Precursor and mature forms of OmpA were separated by SDS-PAGE. (C) Quantitative representations of data from panels A and B. Intensities of full-length translocation products (circles) and the intermediates I_{26} (triangles) and I_{16} (squares) in panel A are plotted against reaction time. Processing efficiencies from panel B are also shown (solid diamonds). Left and right graphs show results with the SecY39-SecE(S105P) IMV and the Sec$^+$ IMV, respectively. (D) Further translocation of I_{16} by a superactive SecA derivative. IMVs from HM808 (*secY39 secE105*) were subjected to a ^{35}S-labeled pro-OmpA translocation reaction at 37°C with wild-type SecA (final concentration, 10 μg/ml) in the absence of the PMF. After 5 min, either SecA329 or wild-type SecA (final concentration, 20 μg/ml) was added to the reaction mixture, followed by further incubation at 37°C. At the indicated time points, samples were treated with proteinase K followed by SDS-PAGE and phosphorimaging. (E) Quantitative representations of the data from panel D. Symbols are the same as in panel C.

transforms the translocation channel into a more relaxed state such that the initial translocation steps are enhanced. However, the SecE(S105P)-altered translocase is less potent in supporting translocation continuation beyond preprotein regions that contain some elements unfavorable to translocation. Thus, the IMV with this altered subunit has a lower overall translocation activity. While we believe that the SecE(S105P) alteration enhances preprotein acceptance, such alteration could well be deleterious to the subsequent continuation and completion processes. A slight defect in translocation was also reported for the *prlG1* mutation (34).

We showed that the SecE(S105P) alteration reduces the interaction between SecY and SecE. This was shown by three lines of evidence. First, the SecYEG complex in the DM-solubilized state dissociates more readily when the SecE subunit contains this amino acid alteration. Second, translocase with both the SecE(S105P) and SecY24 alterations is nonfunctional. The latter result could be interpreted to mean that the combined weakening effects of these mutations on the SecY-SecE interaction are too strong for the double-mutant translocase to maintain the integrity of the translocation channel; the SecY24 alteration weakens the interaction between the cytoplasmic regions of the complex (6), whereas the SecE(S105P) alteration weakens the subunit interaction involving transmembrane regions (12). Third, the mutant cells with the chromosomal *secE105* mutation are no longer interfered with by a range of dominant-negative SecY variants that presumably sequester SecE; in one case, the SecE(S105P) protein interacts differently with the wild-type SecY and the mutant SecY Δ(346-357).

These results suggest that SecY-SecE interaction is crucial for the tuning of the SecYEG channel with respect to its opening to accept the translocation initiation domain of a preprotein. The fact that the SecE(S105P) translocase is more PMF independent than the normal machinery is consistent with the notion that the PMF affects the initiation phase of the translocation reaction (22). In contrast to the SecE(S105P) effect on the PMF dependence of the translocase, the *prlG1* mutant was reported to be hypersensitive to the conditions that lower the PMF level of the cell (27). The latter observation is in accordance with the interfering nature of PrlG1 when expressed in the *secY39* background.

The altered residue, Ser105, is located in the middle of the third transmembrane segment of SecE. The proline substitution for this position may have a major conformational effect. For instance, a kinking of this segment may affect subunit

interactions within the SecYEG complex or interaction between the complexes. Structural and biochemical dissections of the SecYEG complex suggest that an active translocation channel may be formed by superassembly of two or more SecYEG units (7, 39). Driessen and coworkers proposed an intriguing model, that the third transmembrane segment of SecE is tilted, thereby interacting with more than one transmembrane region of SecY and serving as a SecYEG dimer interface (12, 39). It is conceivable that this SecE segment plays important roles in the gating modulation of the translocation channel, and the introduction of proline may affect its transmembrane angle and hence its regulatory function.

We have shown that the SecE(S105P) alteration enhances translocation initiation and makes SecE superactive in overcoming a number of SecY mutational defects. This mutation may provide a useful clue for our further understanding of subunit interactions of the translocase in relation to regulation of the polypeptide-translocating channel.

ACKNOWLEDGMENTS

We thank Naomi Simokawa for construction of plasmid pNA3, Ei-ichi Matsuo for strains and plasmids, and Kiyoko Mochizuki, Mikihiko Yamada, Michiyo Sano, and Yasuhide Yoshioka for technical assistance.

This work was supported by CREST, JST (Japan Science and Technology Corporation) (to K.I.), and grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to H.M. and K.I.).

REFERENCES

1. **Akiyama, Y., and K. Ito.** 1990. SecY protein, a membrane embedded secretion factor of *E. coli*, is cleaved by the OmpT protease *in vitro*. Biochem. Biophys. Res. Commun. **167:**711–715.
2. **Akiyama, Y., and K. Ito.** 1985. The SecY membrane component of the bacterial protein export machinery: analysis by new electrophoretic methods for integral membrane proteins. EMBO J. **4:**3351–3356.
3. **Akiyama, Y., and K. Ito.** 1987. Topology analysis of the SecY protein, an integral membrane protein involved in protein export in *Escherichia coli.* EMBO J. **6:**3465–3470.
4. **Akiyama, Y., T. Ogura, and K. Ito.** 1994. Involvement of FtsH in protein assembly into and through the membrane. J. Biol. Chem. **269:**5218–5224.
5. **Baba, T., A. Jacq, E. Brickman, J. Beckwith, T. Taura, C. Ueguchi, Y. Akiyama, and K. Ito.** 1990. Characterization of cold-sensitive *secY* mutants of *Escherichia coli*. J. Bacteriol. **172:**7005–7010.
6. **Baba, T., T. Taura, T. Shimoike, Y. Akiyama, T. Yoshihisa, and K. Ito.** 1994. A cytoplasmic domain is important for the formation of a SecY-SecE translocator complex. Proc. Natl. Acad. Sci. USA **91:**4539–4543.
7. **Breyton, C., W. Haase, T. A. Rapoport, W. Kuhlbrandt, and I. Collinson.** 2002. Three-dimensional structure of the bacterial protein-translocation complex SecYEG. Nature **418:**662–665.
8. **Duong, F., and W. Wickner.** 1999. The PrlA and PrlG phenotypes are caused by a loosened association among the translocase SecYEG subunits. EMBO J. **18:**3263–3270.
9. **Flower, A. M., R. S. Osborne, and T. J. Silhavy.** 1995. The allele specific synthetic lethality of *prlA-prlG* double mutants predicts interactive domains of SecY and SecE. EMBO J. **14:**884–893.
10. **Homma, T., T. Yoshihisa, and K. Ito.** 1997. Subunit interactions in the *Escherichia coli* protein translocase: SecE and SecG associate independently with SecY. FEBS Lett. **408:**11–15.
11. **Ito, K., D. P. Cerretti, H. Nashimoto, and M. Nomura.** 1984. Characterization of an amber mutation in the structural gene for ribosomal protein L15, which impairs the expression of the protein export gene, *secY*, in *Escherichia coli*. EMBO J. **3:**2319–2324.
12. **Kaufmann, A., E. H. Manting, A. K. J. Veenendaal, A. J. M. Driessen, and C. van der Does.** 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.
13. **Kihara, A., Y. Akiyama, and K. Ito.** 1995. FtsH is required for proteolytic elimination of uncomplexed forms of SecY, an essential protein translocase subunit. Proc. Natl. Acad. Sci. USA **92:**4532–4536.
14. **Kleckner, N. D., D. F. Barker, D. G. Ross, and D. Botstein.** 1978. Properties of the translocatable tetracycline-resistance element Tn*10* in *Escherichia coli* and bacteriophage lambda. Genetics **90:**427–461.
15. **Klionsky, D. J., W. S. A. Brusilow, and R. D. Simoni.** 1984. In vivo evidence for the role of the epsilon subunit as an inhibitor of the proton-translocating ATPase of *Escherichia coli*. J. Bacteriol. **160:**1055–1060.
16. **Manting, E. H., and A. J. M. Driessen.** 2000. *Escherichia coli* translocase: the unraveling of a molecular machine. Mol. Microbiol. **37:**226–238.
17. **Matsumoto, G., H. Nakatogawa, H. Mori, and K. Ito.** 2000. Genetic dissection of SecA: suppressor mutations against the *secY205* translocase defect. Genes Cells **5:**991–999.
18. **Matsumoto, G., T. Yoshihisa, and K. Ito.** 1997. SecY and SecA interact to allow SecA insertion and protein translocation across the *Escherichia coli* plasma membrane. EMBO J. **16:**6384–6393.
19. **Matsuo, E., and K. Ito.** 1998. Genetic analysis of an essential cytoplasmic domain of *Escherichia coli* SecY based on resistance to Syd, a SecY-interacting protein. Mol. Gen. Genet. **258:**240–249.
20. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 431. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Mori, H., and K. Ito.** 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.
22. **Mori, H., and K. Ito.** Biochemical characterization of a mutationally altered protein translocation: proton-motive force stimulation of the initiation phase of translocation. J. Bacteriol. **185:**405–412.
23. **Mori, H., and K. Ito.** 2001. The Sec protein-translocation pathway. Trends Microbiol. **9:**494–500.
24. **Nakatogawa, H., H. Mori, and K. Ito.** 2000. Two independent mechanisms down-regulate the intrinsic SecA ATPase activity. J. Biol. Chem. **275:**33209–33212.
25. **Nichols, B. P., O. Shafiq, and V. Meiners.** 1998. Sequence analysis of Tn*10* insertion sites in a collection of *Escherichia coli* strains used for genetic mapping and strain construction. J. Bacteriol. **180:**6408–6411.
26. **Nishiyama, K., S. Mizushima, and H. Tokuda.** 1993. A novel membrane protein involved in protein translocation across the cytoplasmic membrane of *Escherichia coli*. EMBO J. **12:**3409–3415.
27. **Nouwen, N., B. de Kruijff, and J. Tommassen.** 1996. *prlA* suppressors in *Escherichia coli* relieve the proton electrochemical gradient dependency of translocation of wild-type precursors. Proc. Natl. Acad. Sci. USA **93:**5953–5957.
28. **Osborne, R. S., and T. J. Silhavy.** 1993. PrlA suppressor mutations cluster in regions corresponding to three distinct topological domains. EMBO J. **12:**3391–3398.
29. **Schatz, P. J., P. D. Riggs, A. Jacq, M. J. Fath, and J. Beckwith.** 1989. The *secE* gene encodes an integral membrane protein required for protein export in *Escherichia coli*. Genes Dev. **3:**1035–1044.
30. **Shiba, K., K. Ito, T. Yura, and D. P. Cerretti.** 1984. A defined mutation in the protein export gene within the *spc* ribosomal protein operon of *Escherichia coli*: isolation and characterization of a new temperature-sensitive *secY* mutant. EMBO J. **3:**631–635.
31. **Shimoike, T., T. Taura, A. Kihara, T. Yoshihisa, and K. Ito.** 1995. Product of a new gene, *syd*, functionally interacts with SecY when overproduced in *Escherichia coli.* J. Biol. Chem. **270:**5519–5526.
32. **Shimoike, T., Y. Akiyama, T. Baba, T. Taura, and K. Ito.** 1992. SecY variants that interfere with *Escherichia coli* protein export in the presence of normal *secY*. Mol. Microbiol. **6:**1205–1210.
33. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
34. **Stader, J., L. J. Gansheroff, and T. J. Silhavy.** 1989. New suppressors of signal-sequence mutations, *prlG*, are linked tightly to the *secE* gene of *Escherichia coli*. Genes Dev. **3:**1045–1052.
35. **Taura, T., Y. Akiyama, and K. Ito.** 1994. Genetic analysis of SecY: additional export-defective mutations and factors affecting their phenotypes. Mol. Gen. Genet. **243:**261–269.
36. **Taura, T., T. Baba, Y. Akiyama, and K. Ito.** 1993. Determinants of the quantity of the stable SecY complex in the *Escherichia coli* cell. J. Bacteriol. **175:**7771–7775.
37. **Taura, T., T. Yoshihisa, and K. Ito.** 1997. Protein translocation functions of *Escherichia coli* SecY: *in vitro* characterization of cold-sensitive *secY* mutants. Biochimie **79:**512–517.
38. **van der Wolk, J. P. W., P. Fekkes, A. Boorsma, J. L. Huie, T. J. Silhavy, and A. J. M. Driessen.** 1998. PrlA4 prevents the rejection of signal sequence defective preproteins by stabilizing the SecA-SecY interaction during the initiation of translocation. EMBO J. **17:**3631–3639.
39. **Veenedaal, A. K. J., C. van der Does, and A. J. M. Driessen.** 2002. The core of the bacterial translocase harbors a tilted transmembrane segment 3 of SecE. J. Biol. Chem. **277:**36697–36705.
40. **Yoshihisa, T., and K. Ito.** 1996. Pro-OmpA derivatives with a His_6 tag in their N-terminal "translocation initiation domains" are arrested by Ni^{2+} at an early post-targeting stage of translocation. J. Biol. Chem. **271:**9429–9436.