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Genetics

Residues essential for the function of SecE, a membrane component of the *Escherichia coli* secretion apparatus, are located in a conserved cytoplasmic region

(protein secretion/mutagenesis/SecY)

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ABSTRACT **Protein export in *Escherichia coli* is absolutely dependent on two integral membrane proteins, SecY and SecE. Previous deletion mutagenesis of the *secE* gene showed that only the third of three membrane-spanning segments and a portion of the second cytoplasmic region are necessary for its function in protein export. Here we further define the residues important for SecE function. Alignment of the SecE homologues of various eubacteria reveals that they all contain one membrane-spanning segment, compared with three in *E. coli* SecE, and that the most conserved region among them lies in their putative cytoplasmic amino termini; little homology exists in their membrane-spanning segments. The SecE homologue of the extreme thermophilic bacterium *Thermotoga maritima* was cloned and found to complement a deletion of *secE* in *E. coli*. Deletion or replacement of the cytoplasmic region of *E. coli* SecE eliminated SecE function, indicating that this sequence is essential for a functional secretion machinery. Mutant analysis suggests that the most important function of the third membrane-spanning segment is to maintain the proper topological arrangement of the conserved cytoplasmic domain.**

Six components of the protein export machinery in *Escherichia coli* have been identified through genetic studies (1, 2). The SecB protein is required to maintain a subset of exported proteins in an export-competent conformation in the cytoplasm. The SecA protein, while hydrophilic in nature, is associated with and may even insert into the cytoplasmic membrane (3). Two highly hydrophobic proteins, SecE and SecY, are integral to the cytoplasmic membrane and together form a complex that is essential for the translocation of proteins across vesicle membranes *in vitro* (4, 5; for an alternative finding see ref. 6). The SecD and SecF proteins are also located in the cytoplasmic membrane, but their role is less well studied (7, 8).

Specific roles have been proposed for the membrane-associated Sec proteins. SecA promotes protein translocation by virtue of its ATPase activity (9). SecA is thought to bind to the SecY/SecE complex, which, in turn, may form a pore or channel through which pass the exported proteins (10). Homologues of SecA exist in *Bacillus subtilis* and chloroplasts (11–13). The SecD and SecF proteins may themselves form a complex that acts at a late step in the export process, such as protein release from the membrane (8, 14).

Sequence inspection and topological studies using alkaline phosphatase (PhoA) fusions suggest that SecY contains 10 membrane-spanning segments (MSS; 15) and that SecE contains three MSS (16). Mutant analysis has permitted definition of functional regions of these two proteins (1, 2). The *prlA* alleles of *secY* allow the export machinery to translocate proteins bearing mutant signal sequences and are located in distinct regions of the protein, including its seventh and 10th MSS and first periplasmic loop (17, 18). Important functional domains within SecY have also been inferred from the alignment of numerous homologues identified in mammals, yeast, mycoplasma, archaebacteria, eubacteria, and chloroplasts (19, 20).

Point mutations, deletions, and gene fusions have allowed analysis of the important functional regions of SecE. The *prlG* alleles of *secE*, which permit the export of proteins bearing mutant signal sequences (21), alter residues in either the third MSS of SecE or the periplasmic domain that follows it (Fig. 1*a*). PhoA fusions to the end of MSS3 retain SecE activity, whereas fusions to a position immediately preceding MSS3, or earlier in the protein, do not complement for SecE function (16). Deletion of about two-thirds of SecE (from aa 7–78 out of 127 total), including the first two MSS and a portion of the second cytoplasmic region (Fig. 1*a*), yields a protein that retains some SecE function (23).

Interaction between SecE and SecY has been proposed based on genetic and physiological studies. Synthetic lethality between *prlA* and *prlG* alleles suggests that these proteins form a complex (24). In accordance with this proposal, stability of overexpressed SecY depends on cooverexpression of SecE (25). The region of SecE responsible for the ability to overexpress SecY maps to the carboxyl-terminal portion of SecE and includes MSS3 (26). Taken together, these results suggest that MSS3 and/or the second cytoplasmic domain contain residues important for SecE function, including its interaction with SecY. So far, no point mutations in *secE* that cause severe secretion defects, other than those affecting the level of SecE expression (23), have been detected.

To understand more about the role of SecE in secretion, as well as its potential interaction with other components of the secretion machinery, we have sought to define more precisely the specific regions essential for its function. As one approach, we have identified SecE homologues in other eubacteria and compared them with SecE from *E. coli*. The most apparent amino acid-sequence conservation among the homologues lies within a region corresponding to the second cytoplasmic domain of SecE. Expression of the SecE homologue from *Thermotoga maritima* complements a deletion of the wild-type *secE* gene in *E. coli*. Replacement of MSS3 with a heterologous MSS yields a molecule with SecE function. In addition, we show that mutation of the second cytoplasmic domain renders the protein nonfunctional, suggesting that only this portion of SecE is indispensable for its function in secretion.

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Abbreviations: MSS, membrane-spanning segment(s); PhoA, alkaline phosphatase; ORF, open reading frame.

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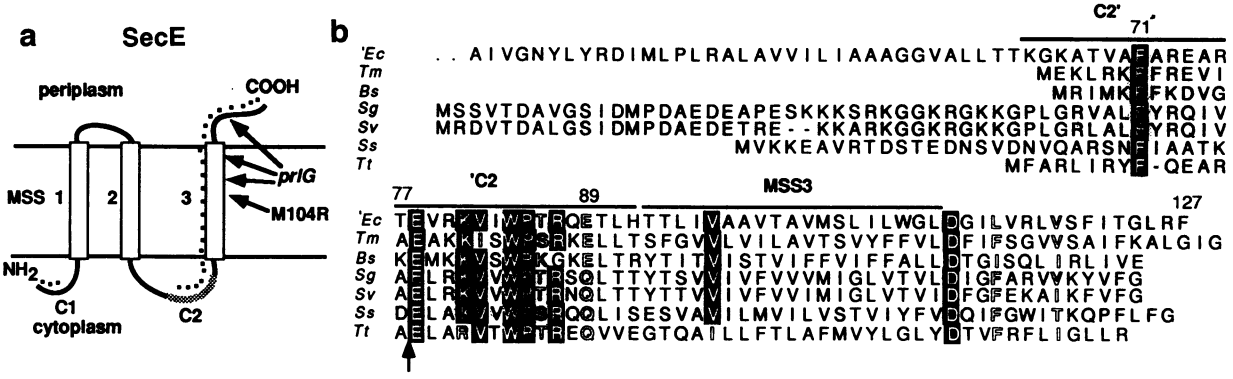

FIG. 1. (*a*) Schematic representation of SecE protein in the cytoplasmic membrane (16), including positions of *prlG* and M104R mutations. The conserved region (aa 71–89) is denoted by the shaded line. Regions contained within the SecEΔ7–78 protein are dotted. (*b*) Alignment of SecE homologues from different species. Amino acid sequences from *E. coli* ('*Ec*; aa 29–127 are shown) SecE, and the *To. maritima* (*Tm*; unclassified, extreme thermophile), *B. subtilis* (*Bs*; Gram-positive), *Streptomyces griseus* (*Sg*; Gram-positive), *Streptomyces virginiae* (*Sv*; Gram-positive), *Synechocystis* species (*Ss*; cyanobacterium), and *Thermus thermophilus* (*Tt*; Gram-negative, thermophile) SecE homologues were aligned by using the CLUSTAL program (PCGene) and visual inspection. The *Tu. thermophilus* homologue was assumed to contain an out-of-frame, upstream Shine–Dalgarno sequence and start codon (22); the reading frame of this start codon and the short peptide following it was shifted −1 by removal of nt 268. The frameshift was placed at a position indicated by the arrow. Absolutely conserved residues are indicated by reverse type, and those residues highly conserved are indicated by outline type. The MSS and putative cytoplasmic (C) regions of SecE and its homologues are indicated by overline. Gaps in alignment are indicated by −. Numbering of positions in sequences is based on the *E. coli* SecE residue number.

MATERIAL AND METHODS

Strains, Plasmids, and Media. *E. coli* strain CM263 was developed for testing the complementing ability of various plasmid-encoded *secE* mutant constructs. CM263 is derived from PS291 [23; *secEΔ19–111, pcnB80 zadL::(Tn10 tet^s str^r), phoAΔPvuII, ΔX74(lac), galE, galK, rpsL(strA), recA::cat*] and contains a complementing copy of *secE* on the pBR-based plasmid pCM10K (unpublished work) instead of pBRU (27). *secE* in pCM10K is flanked by the *rpsL* and *lacY* genes, which confer sensitivity to streptomycin and 2-nitrophenyl β-D-thiogalactoside (28), respectively. Medium (MTSS) to select for CM263 cells that lose pCM10K consists of M63 minimal medium (29) supplemented with 0.4% succinate, streptomycin at 1.5 mg/ml, 1 mM 2-nitrophenyl β-D-thiogalactoside, thiamine at 1 μg/ml, and Casamino acids (0.2%). To test for complementation of Δ*secE*, CM263 was transformed to ampicillin (100 μg/ml) with candidate mutant plasmids, and transformants were then restreaked on MTSS plates and incubated for a minimum of 5 days at 30°C and 37°C to test for growth. CM263 itself, when incubated on MTSS medium, yielded essentially no survivors, because *secE* is essential for cell growth (unpublished work).

pBAD22 is a pBR-derived plasmid encoding *araC* and *bla* and contains the *araBAD* promoter and an optimized Shine–Dalgarno sequence upstream of a multiple cloning site (Luz-Maria Guzman and J.B., unpublished work). pCM22 was constructed by cloning a PCR-generated fragment of *secE* from pJS51 (16) using primers BAD1 (which introduces an *Eco*RI restriction site before the ATG start of *secE*) and T3 into pBAD22. The fragment was cut with *Eco*RI and *Eco*47III and then cloned into *Eco*RI/*Sma* I-cut pBAD22. pCM25 was constructed by cloning a PCR fragment [using primers Toga$_{up}$ and Toga$_{down}$ (see below) and template plasmid PD#968, which contains a 1.5-kb *Eco*RI–*Cla* I fragment encompassing tRNA genes and the *nusG* gene of *To. maritima*] corresponding to nt 835–1035 (30) of the *To. maritima nusG* region into pBAD22. pCM50 was created by first introducing a *Sma* I site at nt 506–511 (27) of pJS51, yielding pJS51S, and then cloning a PCR-generated fragment (using primers FF and secE1) corresponding to nt 137–469 and cut with *Mlu* I into pJS51S that was digested with *Sma* I and *Mlu* I. This technique effectively removed the region encoding aa 77–89 and changed Leu-91 to glycine. pCM60 was constructed in a similar manner, using a PCR fragment encoding the MalF amino-terminal cytoplasmic region (aa 1–19) cloned into a plasmid pJS51NS, which contains an introduced *Nco* I site at nt 427–432. pCM70 was made by first producing "megaprimers" (31) with oligonucleotides secE1 and FE1, and T3 and FE2, using pJS51 as template. These megaprimers were then used to amplify DNA encoding the MalF MSS1 (aa 17–35) and then cloning this construct into pJS51.

The topology of SecE mutant proteins was studied by constructing PhoA fusions to them at a position corresponding to periplasmic aa 119. Construction of the hybrids was done by cloning a *Bst*EII fragment from pJS70 (encoding SecE aa 105–118 fused to PhoA; ref. 16) into *Bst*EII-digested mutant plasmids. Fusion at aa 119 of the wild-type SecE protein results in a hybrid protein that is highly active. Analogous fusions to mutant constructs SecEΔ78–90 and SecE::MalF-N yielded molecules with PhoA activities similar to that of the wild-type SecE::PhoA fusion. The analogous fusion to SecEM104R yielded a hybrid protein that was about half as stable as the wild-type fusion but that had 100-fold less PhoA activity. The stability of the various mutant forms of SecE was assessed by immunoblot analysis of the steady-state levels of the proteins. Anti-SecE serum 91-4 (23) was used for the SecE::MalF-MSS1 construct, and anti-PhoA serum was used for all other constructs.

Sequences of the oligonucleotide primers are as follows: BAD1, 5′-CCGGAATTCTTCTGACAGGTTGGT-3′; T3, 5′-ATTAACCCTCACTAAAG-3′ (anneals downstream of the *Kpn* I site in pJS51); FF, 5′-GTACGCGCTTCACGGG-3′; secE1, 5′-GTTGAGGGCGTATAATCCG-3′; FE1, 5′-GACCTAGCACTGACCAGTGCAATGTTTCCTGGC-3′; FE2, 5′-CCTTGTTGTTTTAATGTACGATGGTATTCTG-GTTC-3′; Toga$_{up}$, 5′-GGAATTCACCATGGAGAAACTC-CGA-3′; Toga$_{down}$, 5′-ACGTTAACCTATTCCCAGCGC-TT-3′; SecE1, 5′-GTTGAGGGCGTATAATCCG-3′; SecE3 (chromosome-specific, ref. 23), 5′-CACGGATTTCAAC-CACTTC-3′. PCR amplification was done with Vent DNA polymerase (New England Biolabs) according to the manufacturer's instructions. When required, PCR fragments were visualized by electrophoresis into 6% nondenaturing acrylamide gels. DNA sequence analysis was performed by using Sequenase (United States Biochemical) according to the manufacturer's instructions. Template DNA was either plas-

mid DNA or agarose (Seaplaque, FMC) gel-separated and purified PCR fragment.

Sequence Acquisition and Alignment. SecE homologues were sought by screening the translated 77th release of GenBank with the peptide FAREARTEVRKVIWPTRQE, corresponding to aa 71–89 of *E. coli* SecE using the TBLASTN alignment search program (32). Computation was done at the National Center for Biotechnology Information using the BLAST network service. High-scoring segment pairs present as open reading frames (ORFs) in regions of the respective genomes corresponding to the *E. coli secE–nusG* locus were considered as encoding homologues of the SecE protein. Only the *Tu. thermophilus* match contained a SecE-homologous ORF with no start codon.

PhoA Assays and Immunoblots. PhoA activity was measured by the method of Derman *et al.* (33). Immunoblots were done by using either anti-SecE 891-4 or anti-PhoA antiserum as described (23).

RESULTS

Comparison of SecE Regions of Various Bacteria. A *B. subtilis* gene that encodes a protein homologous to a part of *E. coli* SecE complements a cold-sensitive mutation in the *secE* gene (34). The *B. subtilis* protein is much smaller than *E. coli* SecE and contains only one MSS. Alignment of the *E. coli* and *B. subtilis* SecE sequences reveals that the homology is most striking between the putative cytoplasmic amino terminus of *Bacillus* SecE (which precedes its sole MSS) and the second cytoplasmic domain of *E. coli* SecE, with only scattered homology throughout the rest of the sequence (Fig. 1*b*). To identify other potential homologues of *E. coli* SecE, the region of homology within the cytoplasmic domain (from aa 71 to 89) was used to search GenBank for similar peptides. A number of ORFs encoding peptides similar to the *E. coli* query sequence were found, including previously unrecognized ORFs in *To. maritima, Streptomyces griseus, Streptomyces virginiae*, and *Synechocystis* sp. These ORFs were located in chromosomal positions corresponding to the *E. coli secE–nusG* and *rplK* loci, suggesting that they code for the SecE homologues from these organisms. Although it has been reported that *Tu. thermophilus* contained no corresponding *secE* gene in this region (22), we found an ORF lacking a start codon but encoding a peptide homologous to SecE upstream of a *nusG* homologue. We suspect that this ORF is, in fact, the *Tu. thermophilus secE* gene for which a translational start site may have been missed because of a sequencing error. In fact, removing nt 268 in the reported nucleotide sequence (22) places an upstream ATG and Shine–Dalgarno sequence in-frame with the SecE-homologous peptide (Fig. 1*b*).

Alignment of the putative SecE homologues was done by using the PALIGN program of PCGene and visual inspection (Fig. 1*b*). Like the *B. subtilis* homologue, each of the other homologues possesses only one region of sufficient hydrophobicity to be a likely MSS, as determined by the HELIXMEM program of PCGene. However, the most conserved sequence lies in a hydrophilic region corresponding to aa 71–89 of *E. coli* SecE: 4 residues are absolutely conserved, and an additional 5 residues are highly conserved. The only other absolutely conserved residue in all of the sequences is an aspartate residue positioned at the carboxyl-terminal end of the putative MSS of the homologues. These MSS are present carboxyl-terminal to the highly conserved region in a position analogous to MSS3 of *E. coli* SecE (Fig. 1).

The *To. maritima secE* homologue complements a deletion of the *E. coli secE* gene. The *To. maritima* SecE homologue is 48% identical in its amino terminus to *E. coli* SecE aa 71–91 and 26% identical in its putative MSS to the *E. coli* SecE MSS3. To determine whether this homologue could complement for SecE function, the putative *To. maritima secE* was cloned into the pBAD22 expression vector (Luz-Maria Guzman and J.B., unpublished work), which places the ORF under control of the *araBAD* promoter (pCM25). As a control, *secE* from *E. coli* was also cloned in the same way into pBAD22 (pCM22). In a wild-type *secE* background, cells containing the plasmid with the *To. maritima secE* gene, but not those containing the parent pBAD22 plasmid or the plasmid with the *E. coli* gene, grew more slowly than wild-type control strains on rich medium, suggesting that the expression of the *To. maritima* protein was somewhat deleterious to the growth of *E. coli*.

Despite the slight inhibition of growth, the cloned *To. maritima* ORF could restore growth to a strain containing a deletion of the chromosomal copy of *secE*. We tested the complementation in a strain (CM263) in which the chromosomal *secE* gene is deleted for sequences encoding nearly the entire SecE protein (*secEΔ19–111*), including its second cytoplasmic domain and three MSS. Because SecE in *E. coli* is indispensable for cell growth (23), the null allele in CM263 was complemented by a counter-selectable plasmid that encodes a kanamycin-resistance gene and a wild-type copy of *secE*. Transformants of CM263 harboring both the counter-selectable plasmid carrying the *E. coli secE* gene and the plasmid carrying the *To. maritima* putative *secE* were plated on medium containing the counterselective agents streptomycin and 2-nitrophenyl β-D-thiogalactoside, either with added arabinose or maltose. CM263 carrying the *To. maritima "secE"* gene under *araBAD* control or the *E. coli secE* gene under *araBAD* control grew as well on this selective medium as a wild-type control strain, but only when the medium contained arabinose (inducing conditions) and not when it contained maltose (noninducing conditions; Table 1). Further evidence that the cells from the arabinose-selective plates had lost the counter-selectable plasmid was that they failed to grow in the presence of kanamycin. As controls, CM263 transformed with a plasmid carrying the *E. coli secE* gene under control of its own promoter grew on either arabinose- or maltose-containing MTSS plates and was sensitive to kanamycin. CM263 harboring pBAD22 failed to grow on MTSS-arabinose or -maltose (Table 1).

To assure that CM263 complemented by the *To. maritima secE* gene contained only that version of *secE*, we employed PCR using various oligonucleotide primers. PCR of whole-cell DNA using a chromosome-specific oligonucleotide revealed that all strains retained the *secEΔ19–111* deletion at the chromosomal locus (data not shown). Cells containing a plasmid-encoded copy of *E. coli secE* all showed an expected 207-bp fragment when *secE*-internal primers were used for PCR. CM263 cells carrying the *To. maritima secE* gene

Table 1. Ability of plasmids to complement a chromosomal deletion of *secE*

Plasmid	Genes	Complement Δ*secE**
pJS51	*secE, bla*	+
pJS51M104R	*secEM104R, bla*	−
pJS82	*secEΔ7–78, bla*	+
pBAD22	*araC, bla*	−
pCM22	*araC, bla, secE*†	+
pCM25	*araC, bla, To. maritima secE*†	+
pCM50	*secEΔ78–90, bla*	−
pCM60	*secE::MalF-N, bla*	−
pCM70	*secE::MalF-MSS1, bla*	+

*Plasmids were transformed into CM263 and then tested for ability to grow on MTSS medium. pBAD derivatives were tested on MTSS medium plus arabinose. +, Growth comparable to that seen in a wild-type strain or CM263/pJS51 at 37°C.

†SecE expressed under control of the *araBAD* promoter.

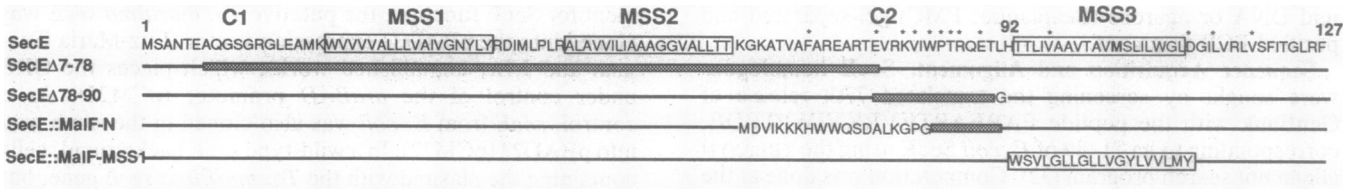

FIG. 2. Deletion and replacement mutants of SecE. The wild-type SecE sequence is denoted at top. MSS are boxed. Asterisks indicate highly conserved residues from Fig. 1*b*. The Met-104 that was mutated to arginine in this study is in boldface type. Sequences deleted in the various constructs are represented by hatched boxes. Amino acid changes are indicated by the amino acid residue substituted at the position, while unchanged amino acids are denoted by solid lines.

lacked this fragment, indicating the lack of a wild-type copy of *secE* (data not shown). We conclude that *To. maritima secE* can functionally replace its *E. coli* homologue.

Mutations Within the Conserved Cytoplasmic Region of SecE. Conservation of residues within the 71–89 region suggests that it may be important for SecE function. To test this, we determined the effect of both removal of this region and its replacement on the ability to complement the chromosomal *secEΔ19–111* deletion. Removal of the conserved region was achieved by creating an in-frame deletion (Fig. 2; pCM50-encoded SecEΔ78–90) of the sequence encoding aa 78–90 in SecE. In addition, the entire second cytoplasmic domain of SecE was replaced with aa 1–19 of the *E. coli* MalF protein (Fig. 2; pCM60-encoded SecE::MalF-N). This region of MalF was chosen because it is localized to the cytoplasm (35) in the native protein and would presumably permit the same topological arrangement of the hybrid protein as is found in SecE. PhoA fusion analysis confirmed that these constructs retained the same topology as native SecE. Immunoblots showed that PhoA fusion protein derivatives of these mutant constructs were as stable as the wild-type fusion, suggesting that the SecE derivatives themselves were stable.

Neither the deletion nor the replacement construct could complement the *secE* null mutation in CM263. These results suggest that residues within the 78–90 region of SecE are necessary for its function.

Replacement of the Third MSS of SecE. We describe evidence in the introduction that MSS3 of SecE contains important structural information. This idea was further supported by the properties of a mutation within this segment (M104R) that disrupted its topology in the membrane. The M104R point mutation replaces a relatively hydrophobic methionine residue in the middle of the MSS with a charged arginine residue (Fig. 2). This mutant form of the protein, expressed from the high-copy plasmid pJS51M104R, was unable to complement the chromosomal *secEΔ19–111* deletion. PhoA fusion analysis indicated that the last periplasmic domain of SecEM104R was, in fact, mislocalized to the cytoplasm, suggesting that MSS3 is not properly integrated into the cytoplasmic membrane in this mutant. Immunoblot analysis of the SecEM104R PhoA fusion protein derivative showed that it was stable.

Although the proper localization of MSS3 to the cytoplasmic membrane seems important for SecE function, only one of the highly conserved residues among the homologues lies in this MSS (Fig. 1*b*). This finding raises the possibility that MSS3 serves merely to anchor the essential cytoplasmic domain preceding it to the inside face of the cytoplasmic membrane. To address this possibility, we examined a mutation, SecE::MalF-MSS1, that replaces SecE MSS3 with MSS1 (aa 17–35; ref. 36) of the MalF protein (Fig. 2; encoded on pCM70). If MSS3 of SecE serves merely as an anchor sequence, MSS1 of MalF should also provide this function and allow the resulting hybrid protein to complement for SecE function. This construct was able to complement the *secEΔ19–111* chromosomal deletion at 37°C but complemented poorly at 42°C or 30°C. The inability of the M104R mutation to complement combined with the complementation observed with the SecE::MalF-MSS1 replacement suggests that an important role for MSS3 is to anchor the second cytoplasmic domain to the cytoplasmic membrane.

DISCUSSION

Previous genetic analysis of SecE has shown that a region that includes MSS3 and that extends from the middle of the second cytoplasmic domain to a position in the second periplasmic domain is sufficient for its function in protein export (2, 23). The *prlG* point mutations in MSS3 and in the second periplasmic domain also point to these regions of the protein as being important for function (21). The results we present here further define residues within SecE that are indispensable for its function in secretion.

Our first indication of essential SecE residues came from a comparison of the *E. coli* SecE protein to apparent homologues in other bacteria (Fig. 1*b*). These homologues were detected mainly because they occur in the same position relative to homologues of the *E. coli nusG* gene as the *secE* gene does in *E. coli*. One of the homologues (from *To. maritima*) is, in fact, a functional homologue, as demonstrated by its ability to substitute for the *E. coli secE* gene. Comparison of these "SecE" sequences reveals a motif encompassing 19 aa. Nine of 19 aa are well conserved in the region corresponding to aa 71–89 of *E. coli* SecE second cytoplasmic domain (Fig. 1). Replacement or deletion of most of this region of SecE leads to loss of function (Table 1). We show that the failure of these constructs to complement a *secE* null mutation cannot be attributed to instability or alteration of the membrane topology of the gene products.

Our sequence comparisons also show that all SecE homologues from other bacteria contain one region of sufficient length and hydrophobicity to span a membrane in an α-helical conformation, whereas *E. coli* SecE possesses three MSS. Three pieces of evidence suggest a possible function for SecE MSS3. (*i*) Silhavy and colleagues (21) have isolated *prlG* mutations in the *secE* gene. These mutations, when present in strains containing certain *prlA* alleles, impart a severe growth defect, suggesting an interaction between MSS3 of SecE and MSS10 of SecY that stabilizes the SecE/SecY complex. (*ii*) We have found that replacement of MSS3 of SecE with MSS1 of MalF results in SecE that is functional but also temperature-sensitive and cold-sensitive. In addition, the *To. maritima* SecE clone, in which the MSS is only 26% similar to that of *E. coli*, could complement for SecE function. Thus, although there may be an interaction or apposition of MSS3 of SecE with some portion of SecY, the specificity of this interaction is not essential for functioning of the complex. (*iii*) The proper integration of MSS3 appears necessary for SecE function, as shown by the inability of the topologically altered SecE-M104R construct to complement for SecE function.

What functions might be served by the second cytoplasmic domain of SecE? Various lines of evidence suggest that SecE must interact with SecY. In addition, it has been proposed that SecE and SecY interact with the SecA protein (37, 38).

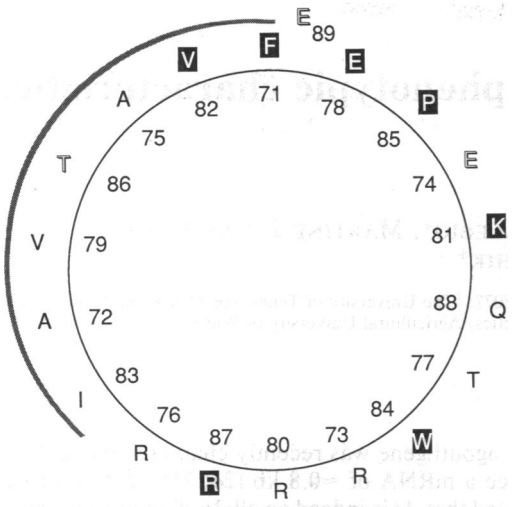

FIG. 3. Helical wheel representation of the region containing conserved residues in C2 of SecE. Absolutely conserved residues are denoted in reverse type, and those highly conserved are indicated in outline type. Amino acid numbers are given inside the wheel. The hydrophobic face of the helix (see text) is overlined with a shaded bar.

While we have observed here a strongly conserved motif across species for SecE, strongly conserved motifs also exist in SecA and SecY homologues (see the introduction). Thus, this cytoplasmic domain could be important for such interactions. Alternatively, this domain may serve as a site of modification by a cytoplasmic enzyme or as a site of interaction with some other cytoplasmic component. Proper localization of the second cytoplasmic domain of SecE to the cytoplasmic face of the cytoplasmic membrane is necessary for its function (see above). Additional features of this domain may contribute to its proper functional orientation. When the residues from the conserved cytoplasmic region of SecE are arranged in a helical wheel plot, they form an amphipathic helix (Fig. 3). If one envisions this helix lying on the inner surface of the cytoplasmic membrane, then it becomes apparent that the most conserved residues are clustered on its hydrophilic face. Perhaps this arrangement "points" the side chains into the cytoplasmic milieu, where they are available for interaction or modification. Our mutations that alter the cytoplasmic domain of SecE should be useful for testing the proposals for its interaction with other proteins.

In summary, SecE contains certain elements, such as MSS3, that are responsible for its overall structure and topology. These elements may also contribute to the stability of complexes between SecE and other members of the secretion apparatus. The absolute requirement of residues within the second cytoplasmic domain points to this portion of the molecule as crucial to its function in secretion.

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