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Cloning and molecular characterization of the *secY* genes from *Bacillus licheniformis* and *Staphylococcus carnosus*: comparative analysis of nine members of the SecY family

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Summary. SecY is a central component of the export machinery that mediates the translocation of secretory proteins across the plasma membrane of *Escherichia coli*. We have cloned and sequenced the *secY* genes from *Bacillus licheniformis* and *Staphylococcus carnosus*. The deduced amino acid sequences are highly homologous to those of other known SecY polypeptides, all having the potential to form 10 transmembrane segments. Comparative analysis of 9 SecY polypeptides, derived from different bacteria, revealed that 14 amino acid positions (2,7%) are identical in all SecY proteins and 89 (16.9%) show conservative changes. Clusters of conserved amino acid residues were found in 4 of the 10 transmembrane segments and 2 of the 6 cytoplasmic domains. It is suggested that the conserved regions might be involved in the translocation activity of SecY or might be required for the correct interaction of SecY with other components of the secretion apparatus.

Key words: Gram-positive bacteria – Protein secretion – Membrane protein – SecY – Nucleotide sequence

Introduction

Together with SecA (Oliver et al. 1990), SecB (Kumamoto et al. 1989), SecE (Riggs et al. 1988; Schatz et al. 1989), SecD and SecF (Gardel et al. 1987; 1990), the SecY protein (Ito 1984) forms part of the translocation machinery which mediates the transport of proteins across the cytoplasmic membrane of *Escherichia coli* (Wickner et al. 1991). Genetic (Bieker and Silhavy 1990) and biochemical (Nishiyama et al. 1991) analyses have shown that the SecY protein has a central role in the secretory pathway and, together with the SecE protein, is sufficient to allow SecA-dependent translocation of precursor proteins in reconstituted systems (Brundage et al. 1990; Akimaru et al. 1991).

SecY is an integral protein of the *E. coli* plasma membrane which includes 10 transmembrane segments (Akiyama and Ito 1987). Mutations have been identified in the *secY* gene (Cerretti et al. 1983) which result either in defective precursor translocation (Shiba et al. 1984; Baba et al. 1990; Ito et al. 1989) or suppress certain defects in the signal sequence of exported proteins (Emr et al. 1981; Sako and Iino 1988; Sako 1991). Since these mutations have been found in various regions of the *secY* gene and because long-range conformational effects exerted by these mutations cannot be ruled out, additional approaches to the elucidation of structure-function relationships in the SecY polypeptide would be desirable.

In the case of outer membrane protein OmpA, the comparative analysis of amino acid sequences of OmpA proteins, derived from various bacteria, has contributed significantly to the understanding of the structural organization of the OmpA protein in the outer membrane (Braun and Cole 1984) and has led to the identification of functional domains (Cole and Maldener 1986). Therefore, a similar approach might be very useful for the characterization of the SecY protein at a molecular level. Genes that code for proteins highly homologous to the *E. coli* SecY polypeptide have recently been identified in *Bacillus subtilis* (Suh et al. 1990; Nakamura et al. 1990), *Lactococcus lactis* (Koivula et al. 1991), *Micrococcus luteus* (Ohama et al. 1989), *Mycoplasma capricolum* (Ohkubo et al. 1987), *Cyanophora paradoxa* (Michalowski et al. 1990) and *Methanococcus vanniellii* (Auer et al. 1991).

Certain gram-positive bacteria are widely used for the industrial production of extracellular enzymes (Priest 1977). However, in comparison with the situation in *E. coli* much less is known about the export apparatus in these organisms (Freudl 1992). In this communication, we describe the cloning and nucleotide sequences of *secY* genes from the gram-positive bacteria *Bacillus licheniformis* and *Staphylococcus carnosus*. Alignment of 9 different SecY polypeptides allowed the identification of regions which have been highly conserved during evolution.

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Materials and methods

Bacterial strains, phages and media. Strains were grown in Luria broth (LB) or on Luria agar (Miller 1972) supplemented with 40 µg ampicillin/ml, 30 µg kanamycin/ml, 30 µg chloramphenicol/ml, 0.5% (w/v) glucose, or 1 mM isopropylthiogalactoside (IPTG), as required. *B. licheniformis* DSM13 (Smith et al. 1964), *S. carnosus* TM300 (Schleifer and Fischer 1982) and *B. subtilis* DB104 (Kawamura and Doi 1984) were used as sources for the isolation of chromosomal DNA. Genomic libraries were constructed in the bacteriophage vector lambda gt11 (Huynh et al. 1984) and plated onto *E. coli* Y1090 cells (Young and Davis 1983).

Plaque hybridization and Southern blotting. A 1 kb *Sma*I-*Eco*RV fragment of pST120 (Suh et al. 1990), which comprises almost the entire *secY* gene of *B. subtilis*, was labeled with digoxigenin using a non-radioactive DNA labeling and detection kit (Boehringer Mannheim) according to the instructions of the manufacturer. Plaque hybridization and Southern analysis were performed as described previously (Overhoff et al. 1991).

DNA techniques. Isolation of chromosomal DNA, preparation of plasmid and phage lambda DNA and other DNA techniques followed standard procedures (Maniatis et al. 1982). The genomic libraries were constructed by cloning 2–5 kb chromosomal DNA fragments, prepared by *Eco*RI digestion, in the *Eco*RI-digested bacteriophage vector lambda gt11. The ligation mixture was packaged in vitro (Hohn 1979) and used to infect *E. coli* Y1090. From the isolated recombinant bacteriophages, *Eco*RI fragments of either 4.1 kb (*B. licheniformis*) or 2.9 kb (*S. carnosus*) were subcloned in the *Eco*RI-digested plasmid vector pBGS18 (Spratt et al. 1986), yielding pBL43 (containing the *B. licheniformis secY* gene) and pSC45 (containing the *S. carnosus secY* gene). For DNA sequencing the chain termination method (Sanger et al. 1977) was used.

Computer analysis of sequence data. Nucleotide and amino acid sequences were analyzed with the PC/Gene software package (IntelliGenetics, Mountain View, Calif.). Alignment of the *SecY* amino acid sequences was done using the CLUSTAL program of Higgins and Sharp (1988).

Results

Cloning of DNA fragments from *Bacillus licheniformis* and *Staphylococcus carnosus* which hybridize to the *Bacillus subtilis secY* gene

Chromosomal DNA from *B. licheniformis* and *S. carnosus* was analyzed by Southern hybridization using a 1 kb *Sma*I-*Eco*RV fragment of pST120, which includes almost the complete *secY* gene from *B. subtilis* (Suh et al. 1990), as a probe. In the *Eco*RI digest of *B. licheniformis*

Fig. 1. Identification of *secY* in *B. licheniformis* and *S. carnosus* by Southern blotting. *Eco*RI-digested chromosomal DNA from *B. subtilis* DB104 (lane 2), *B. licheniformis* DSM13 (lane 3), *S. carnosus* TM300 (lane 4), or plasmid pST120 (lane 1) were electrophoresed on a 0.7% agarose gel, blotted onto nitrocellulose filters and hybridized with the digoxigenin-labelled 1 kb *Sma*I-*Eco*RV fragment of pST120. Detection of reactive bands was by an enzyme-linked immunoassay using anti-digoxigenin antibody which has been conjugated to alkaline phosphatase. Numbers at the left margin indicate the size (in kb) of the markers used

chromosomal DNA, a 4.1 kb fragment was detected which hybridized to the *B. subtilis secY* gene. Likewise, a 2.9 kb fragment was detected in the *Eco*RI-digested *S. carnosus* chromosomal DNA (Fig. 1). Therefore, for the construction of suitable genomic libraries, chromosomal DNA from *B. licheniformis* and *S. carnosus* was digested with *Eco*RI, and DNA fragments, 2–5 kb in size, were isolated and ligated into the *Eco*RI-digested cloning vector lambda gt11. Several recombinant phages showing a positive signal in a plaque hybridization assay were identified in both the *B. licheniformis* and the *S. carnosus* genomic libraries. The isolated phages contained *Eco*RI inserts of 4.1 kb (*B. licheniformis*) or 2.9 kb (*S. carnosus*) in size. Subcloning of the corresponding fragments in the plasmid vector pBGS18 (Spratt et al. 1986) resulted in plasmids pBL43 (containing the *B. licheniformis* DNA fragment) and pSC45 (containing the *S. carnosus* DNA fragment).

Nucleotide sequences of the *B. licheniformis* and *S. carnosus secY* genes

The nucleotide sequence of a 1.65 kb DNA fragment from pBL43, encompassing the *B. licheniformis secY* gene, is shown in Fig. 2. An open reading frame (ORF) of 1293 bp was detected, starting at position 221 (Fig. 2). A potential TTG start codon is preceded by a possible ribosome-binding site (GAGG; positions 208–211). In *B. subtilis*, the *secY* gene is part of the *spc* operon and is preceded by the gene coding for ribosomal protein L15 and followed by the *adk* gene (Suh et al. 1990; Nakamura et al. 1990). The same genomic organization was found for the *B. licheniformis secY* gene. In this case also, the *secY* gene is preceded by the gene coding for L15 and followed by *adk*. Since no potential promoter sequences can be identified upstream of the *B. licheniformis secY*

10 20 30 40 50 60
 GCTGTGGTCAACCTAGACAACTCAACAGCTTTGCAGAGGGACGGAAGTTACTCTCTGAA
 A V V N L D K L N S F A E G T E V T P E
 CTTCTTTTAGAACAGGTGTAATCAGCAAGTTGAAATCTGGAGTGAATAATCTTGGCGAC 120
 L L L E T G V I S K L K S G V K I L G D
 GGTAAATAGAAAAGAAATTAAGTGTAAAGCCAATAATCTCCGCTTCTGCTAAACAA 180
 G K L E K K L T V K A N K F S A S A K Q
 GCAATGAGGCTGCTGGCGTACAGCTGAGGTGATCTAACTTTGTTAAGACAATCTCCAA 240
 A I E A A G G T A E V I - M F K T I S N
 SecY ▶
 TTTTATGCGTGTGAAGGATATTCGGAATAAGATATCTTCACTCTTTAATGCTTATCGT 300
 F M R V K D I R N K I I F T L L M L I V
 CTTTCGTCTCGGTTCGTTTCATCTCTGTCGCGCAGTAAACACAGAAGTTTAAAGGCACA 360
 F R L G S F I P V P H V N T E V L K A Q
 GGATCAATGAGCGTATTGGGATTCTAAACACATTTGGCGCGGTCATTGTCTCAACTT 420
 D Q M S V F G I L N T F G G A L F N F
 CTCCTATCTTGGGATGGGAATCATGCTTACATCACAGCTTCTATCATCATCCAGCTCCT 480
 S I L A M G I M P Y I T A S I I I Q L L
 GCAGATGGATGTTGTCGCGAAGTTTACCGAATGGTCTAAGCAGGGGAAGTTGGTCGCG 540
 Q M D V V P K F T E W S K Q G E V G R R
 AAACTAGCTCAGTTTCAAGTACTTACAGTCTGTTTGGATTATCCAGGCTTTAGG 600
 K L A Q F T R Y F T I V L G F I Q A L G
 TATGCTTACGGGTTTAAACAACATGGCGCGCGCGCTTATTACAGACCGGAGTGG 660
 M S Y G F N N M A G G A L I T D P G V G
 TACTTATTGCTTATTGCGATTGTACTAAGTCTGGAAGTCTTTTAAATGTCGCTGG 720
 T Y L L I A I V L T A G T A F L M W L G
 AGAACAATACGCTCTCATGGAGTTGGTAACGAATATCCATATCATCTTCGCGGTAT 780
 E Q I T S H G V G N G I S I I I F A G I
 CGTTGCGGTATTCGCGAGACGATTATACAGTCTACGCTCAGCAGTTTGTAGATGCTG 840
 V A G I P Q T I N Q I Y A Q Q F V D A G
 AGATCAGTTATCTTGCAGATCATAAGGTTGCTTATATGGTTGCGATTATTAGCGAT 900
 D Q L F L Q I I K V V V I L V A I L A I
 TGTCTGTCGCTTATTTTCATCAACAGCGGTTAGAAAAATCTCAATCAATATGCGAA 960
 V V G V I F I Q Q A V R K I S I Q Y A K
 AGGTTACGCGCTTACCGGTTCTTGGCGCCAGTCTACACACTTGGCGCTGAAGGTTAA 1020
 G S G R S P V P G G Q S T H L P L K V N
 CCCGCGGGAGTTATTCGCTTATCTTGGCGTAGCATTATCACCACTCCGCGGACCGT 1080
 P A G V I P V I F A V A F I T T P R T V
 GGCACATCTTCTTGGCTCAATGACGTCACGAATGGATTCAAAAAACGTTTGTATACAC 1140
 A T F F G S N D V T N W I Q K T F D Y T
 GCATCCGCTCGGAATGGGAGTTTATGCGAGCATGATCATCGCTTTACCTATTTCTATGC 1200
 H P V G M G V I A A L I A F T Y F Y A
 ATTTGTCAGGTTTAACTTACGCAATGGCGGATAATCTTAAAAACAGGTGGCTATAT 1260
 F V Q V N P E Q M A D N L K K Q G G Y I
 TCCGGGAGTTCTGTCAGGAAAAATGACTCAAGATAGATTACGACATTTGTATCGACT 1320
 P G V R P G K M T Q D R I T S I L Y R L
 TACATTGTAGGCTCCATATCTTGGCGGTGATTGCCATCTTCTCTGCTTGTCTGTTAA 1380
 T F V G S I F L A V I A I L P V L F V N
 TATTGCTGGGCTGCTTCTTCTGCAACAATGGTGAACAAGTTTGTGATTTGTCATCGG 1440
 I A G L P S S A Q I G G T S L L I V I G
 GGTTCGACTTGAACAATGAAGAGCTTGAAGCCAGCTGGTAAACGAACTATCGTGG 1500
 V A L E T M K Q L E S Q L V K R N Y R G
 ATTTATGAAGCACTAGAGGAAATGGGCGCGCCATTCCTCTAAAAATAGAGGACGGG 1560
 F M K H -
 GAATCCGAAATGAAGTCTTAACTGGGACTGCTGGAGCGGTAAAGGACACAGGCT 1620
 M N L D L M G L P G A G K G T Q A
 GAACGGATTGTTGAAGATTT
 E R I V E D F

Fig. 2. Nucleotide and predicted amino acid sequences of a 1.64 kb DNA fragment from the *B. licheniformis* *secY* region. Nucleotides are numbered from the 5' end. The putative ribosome-binding site for the *secY* gene is underlined

10 20 30 40 50 60
 CAGACAGCTTGTAACTTAGAACAACTAAACAGATTGGAAGAAGGTACAGAAGTTACTCC
 R Q L V N L E Q L N R F E E G T E V T P
 TGAGCTTTTAATCGAACTGGTGTGTTAAAAACGCTAAAGCGGTATCAAGATTAGG 120
 E L L I E T G V V K N A K A G I K V L G
 TAATGGTCTCTAGAGAAAAATTAACAGTAAAGCGCATATTTCTCTGCATCTGCAGC 180
 N G S L E K K L T V K A H N F S A S A A
 TGAAGCAATTGATGCTAAAGGTGGAGCACAGGTTGATCTAATGTTGAAACAATGTT 240
 E A I D A K G G A H E V I - M F E T I V
 SecY ▶
 AACTTCTTAAACTAAAGAAGTACGGAACAGATCTTTTACGCTCGCAATGCTAGTT 300
 N F F K T K E V R N K I F F T L M A L V
 ATTTTAAATAGTACTTATATACCTGCGCTGGAGTCAATCCAGCTGCATTTGATAAC 360
 I F K I G T Y I P A P G V N P A A F D N
 AATCAAGGTTCTCAAGGTGCTAGTATTTATTAATACTTTTGGTGGCGGACCTTGAAG 420
 N Q G S Q G V T D L L N T F G G G A L K
 AACTTTCCATATTGCAATGGGTATCATGCCCTACATCACTGCTTCAATCGTAATGCAG 480
 N F S I F A M G I M P Y I T A S I V M Q
 TTATGCAATGATATTGTTCCGAAATTACAGAATGGCTAAACAGGGTATGTTGGA 540
 L L Q M D I V P K F T E W A K Q G D V G
 CGTAAGAAATTAATAACGTTACAGTATTTTGGCATTATCTTACGCTTATCCAACTCC 600
 R K K L N N V T R Y F A I I L A F I Q S
 ATCGGTATGGCATCCAGTTTAACTATTTGAAGGGCGCATGATTATGATCTCTCT 660
 I G M A F Q F N N Y L K G A L I I D P S
 CCGATGAGTTATTTGTTAATGCTATTGATTAACAACGTTACTGCTATCTTATATGG 720
 P M S Y L L I A I V L T T G T A F L L W
 TTAGGTGAACAACTACTCAGTATGGTGTAGTAAATGGTATTTCAATCATATCTTTGCT 780
 L G E Q I T Q Y G V G N G I S I I I F A
 GGTATTTGTCACATTGCCATCATCGCTTATTCAGTTTATCAACAAGCCTTTGTCGGG 840
 G I L S T L P S S L I Q F Y Q Q A F V G
 CAAAGTGATACATCTATGCGATGGTTGCAAGTTGCTGGTTAGTTATGTTAGTACTA 900
 Q S D T S M A W L Q V A G L V I G L V L
 TTGACTATGGGTGCTGTTTACGTACTTCAAGCTGACGTAATAATTCATCAATATGCT 960
 L T M G A V Y V L Q A V R K I P I Q Y A
 AAAAGCAATCTACTCAGCGTTTAGGATCAATGCAAGCTATTTCGCGTTAAAGTTAAC 1020
 K K Q S T Q R L G S N A T Y L P L K V N
 TCAGCAGGGTTATCCAGTTATCTTCGCAATGGCATCTTCTTGTGCTAGAACATTA 1080
 S A G G V I P V I F A M A F F L L P R T L
 ACAATGTTCTTCCCTAAAGCAGATTGGGCTCAGCAAAATGCTAATACAGCAATCCATCA 1140
 T M F F P K A D W A Q Q I A N T A C A N P S
 AGTAATATCGGAATGGTAATTTATATCTTGATCATTCGATTACTTACTTCTATGCA 1200
 S N I G M V I Y I I L I I A F T Y F Y A
 TTTGTTCAAGTTAATCCTGAAAAATGCTTGACAACTTAAAGAACAGGCAGTTACGTT 1260
 F V Q V N P E K M S D N L K K Q G S Y V
 CCAGGTATTAGACCTGGTGAACAACTAAAGATATTTACTAAAGTTTATATCGTTTA 1320
 P G I R P G E Q T K K Y I T K V L Y R L
 ACTTTGTTGGTTCTATATCTTGGCAGTTATCGCTATCTTGGCGATACGCTACTAAG 1380
 T F V G S I F L A V I A I L P I L A T K
 TTTATGAACCTACCGCAGTCAATCCAGTCGCGGTACAAGCTTATTAATCGTTATCGGT 1440
 F M N L P Q S I Q V G G T S L L I V I G
 GTTGAATCGAAACGATGAAGATTGGAAGCGCAAGTTAAACAAAAGATATAAGGC 1500
 V A I E T M K S L E A Q V N Q K E Y K G
 TTTGGTGTAGATAAAGTGTAGAGGCAATATGAATATCATCTTAATGGGTTTACCTGG 1560
 F G G R - M N I I L M G L P G
 TGCAGGTAAAGGAAGTCAAGCGAGTGAATAGTTAAGAAATTCCTATTCGCGATATATC 1620
 A G K T T Q A S E I V K K F P I P H I S
 TACAGGTGATATGTTTATAAAGCTATCAAA
 T G D M F I K A I K

Fig. 3. Nucleotide and predicted amino acid sequences of a 1.65 kb DNA fragment from the *S. carnosus* *secY* region. Nucleotides are numbered from the 5' end. The putative ribosome-binding site for the *secY* gene is underlined

gene, it seems very likely that the *secY* gene is transcribed from the *spc* promoter.

The nucleotide sequence of the *S. carnosus* *secY* region is shown in Fig. 3. An ORF of 1290 bp, starting with an ATG codon at position 223, is preceded by a potential ribosome-binding site (GAGG; position 212–215). As in the case of *B. licheniformis*, the *S. carnosus* *secY* gene is

flanked by sequences coding for the homologues of the ribosomal protein L15 and adenylate kinase. Here again no sequences which might constitute a potential promoter could be identified upstream of the *S. carnosus secY* gene.

Table 1. Degrees of relatedness of SecY proteins from various bacteria

	BL	BS	SC	LL	EC	MC	ML	CP	MV
BL	-	91.4	75.5	69.6	67.6	68.2	66.0	60.0	56.2
BS	84.0	-	75.3	69.2	65.0	65.6	66.9	61.4	54.8
SC	59.4	60.3	-	67.6	65.6	66.3	66.9	62.1	51.9
LL	47.8	48.0	45.6	-	64.0	66.6	63.2	59.5	54.0
EC	41.8	41.1	41.2	38.0	-	61.2	65.2	58.2	50.6
MC	39.7	39.2	39.8	39.0	33.0	-	60.9	57.4	54.5
ML	37.6	38.3	38.6	33.7	38.4	25.9	-	58.3	50.6
CP	29.5	28.8	30.5	30.3	26.2	26.1	28.7	-	52.4
MV	19.5	22.3	21.3	20.0	20.1	20.1	20.7	18.1	-

Values below the diagonal represent percentage of identical amino acid residues; values above the diagonal represent the percentage of identical plus chemically related amino acids. Species abbreviations as in Fig. 4

Sequence alignment of a family of SecY proteins

The SecY proteins of *B. licheniformis* (431 amino acid residues) and *S. carnosus* (430 amino acid residues) show a high degree of similarity to the SecY polypeptides from *E. coli* (Cerretti et al. 1983), *B. subtilis* (Suh et al. 1990; Nakamura et al. 1990), *L. lactis* (Koivula et al. 1991), *M. luteus* (Ohama et al. 1989), *M. capricolum* (Ohkubo et al. 1987), *C. paradoxa* (Michalowski et al. 1990) and *M. vannielii* (Auer et al. 1991) (Table 1). The *E. coli* SecY protein is thought to contain 10 transmembrane segments (TMS; Akiyama and Ito 1987). Prediction of the hydropathy profiles for the *B. licheniformis* and *S. carnosus* SecY proteins (not shown) and alignment of the sequences with those of other members from the SecY family indicated that the transmembrane topology might be the same for all SecY polypeptides (Fig. 4). Furthermore, the alignment of all 9 SecY proteins showed that, at the corresponding amino acid positions, 14 amino acid residues (2.7%) are strictly conserved and 89 (16.9%) positions show conservative changes (Fig. 4). The most conserved regions were found in TMS 2, 5, 9 and 10 and in the hydrophilic loops connecting TMS 4 with 5 and TMS 8 with 9. Interestingly, except for the *secY100* mutation, in which a glycine residue is changed to glutamic acid (Ito et al. 1989), none of the known *secY/prlA* mutations affects an amino acid residue that has been highly conserved.

Discussion

In *E. coli*, the SecY protein is of crucial importance for the translocation of secretory proteins across the cytoplasmic membrane (Ito et al. 1983) and, together with the SecE protein, forms part of the membrane-bound translocator complex (Bieker and Silhavy 1990; Brundage et al. 1990). However, the precise role of the SecY protein in protein translocation at the molecular level is completely unknown. The comparative analysis of SecY proteins, derived from different bacteria, therefore represents a useful approach for the identification of functionally important regions. In this communication, we

describe the cloning and nucleotide sequencing of the *secY* genes from the industrially important gram-positive bacteria *B. licheniformis* and *S. carnosus* and the comparison of their deduced amino acid sequences with those of other proteins from the SecY family.

As in the case of *B. subtilis*, the *secY* genes from *B. licheniformis* and *S. carnosus* are located within the ribosomal *spc* operon and are preceded by the gene coding for ribosomal protein L15 and followed by the *adk* gene (Suh et al. 1990; Nakamura et al. 1990). Since no obvious promoter sequences could be found upstream of the *secY* genes in either case, transcription of the *secY* genes most probably occurs from the promoter of the *spc* operon. The deduced amino acid sequences of the SecY proteins from *B. licheniformis* and *S. carnosus* show substantial similarities to other SecY polypeptides. The *E. coli* SecY protein is thought to span the cytoplasmic membrane 10 times (Akiyama and Ito 1987). Analysis of the hydropathy profiles and alignment of all known SecY sequences reveal that this membrane topology may well be conserved in all SecY polypeptides examined so far. In all cases, 10 potential transmembrane segments could be identified. However, clusters of highly conserved amino acid residues are found in only four of the transmembrane segments (Fig. 4; TMS 2, 5, 9, 10). It is tempting to speculate that these regions might be directly involved in the translocation activity or might be required for the proper interaction of SecY with other membrane components of the translocation apparatus. In addition, the hydrophilic loops connecting TMS 4 with 5 and TMS 8 with 9, which, according to the topological model for the *E. coli* SecY polypeptide (Akiyama and Ito 1987), face the cytosol, are remarkably conserved. Because SecA, a peripheral plasma membrane protein (Oliver et al. 1990) and SecY are thought to interact (Fandl et al. 1988; Hartl et al. 1990), these cytosolic loops could be domains which are involved in SecA/SecY interaction. Except for one mutation (*secY100*), the *E. coli secY/prlA* mutations isolated so far do not affect strictly conserved amino acid residues. This might suggest that the conserved residues are directly involved in the proper functioning of SecY as part of the protein translocator complex. Mutations which alter structurally or functionally important amino acid residues might cause the complete loss of SecY function and, due to the essential nature of SecY activity (Ito et al. 1983; Nishiyama et al. 1991), would not have been recovered in the selection procedures used so far. Site-directed mutagenesis procedures may now be used to test the involvement of the corresponding amino acid residues in SecY function in a more direct manner.

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