Deregulation of the SecYEG Translocation Channel upon Removal of the Plug Domain*

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Previous studies have shown that the SecY plug is displaced from the center of the SecYEG channel during polypeptide translocation. The structural and functional consequences of the deletion of the plug are now examined. Both in vivo and in vitro observations indicate that the plug domain is not essential to the function of the translocon. In fact, deletion of the plug confers to the cell and to the membranes a Prl-like phenotype: reduced proton-motive force dependence of translocation, increased membrane insertion of SecA, diminished requirement for functional leader peptide, and weakened SecYEG subunit association. Although the plug domain does not seem essential, locking the plug in the center of the channel inactivates the translocon. Thus, the SecY plug is important to regulate the activity of the channel and to confer specificity to the translocation reaction. We propose that the plug contributes to the gating mechanism of the channel by maintaining the structure of the SecYEG complex in a compact closed state.

The Sec translocon is a universally conserved membrane protein complex that cooperates with cytosolic partners to transport proteins into or across membranes. The membrane core complex (SecYEG in bacteria) forms the conduct for polypeptides. Membrane proteins are mostly inserted co-translationally by SecYEG-bound ribosomes, whereas secretory precursors are translocated post-translationally by the motor ATPase SecA (1–4). In bacteria, the translocation process is enhanced by the proton gradient across the inner membrane, the proton motive force (PMF) (5).

The crystal structure of the Methanococcus jannaschii SecY complex (6) has unraveled the architecture of the core translocon. It consists of four key structural elements: the “channel,” located in the body of the SecY subunit; the “pore ring” made of 6 residues forming a constriction point in the middle of the channel; the “plug” domain formed by a small helix seating on top of the constriction on its periplasmic side; and the “lateral gate” made by the juxtaposition of two SecY transmembrane (TM2 and TM7) segments that create an opening toward the lipid bilayer. The pore ring and the plug domain were proposed to close and to seal the channel, whereas the lateral gate would serve to accommodate the leader peptide of the preprotein or to release membrane protein domains into the membrane (6, 7).

Increasing experimental evidences support the model predicted from the crystal structure. Using a photo-cross-linking approach, it was demonstrated that the leader peptide contacts the two TM segments forming the lateral gate (8). Using disulfide cross-linking techniques, it was shown that the plug moves away from the center of the channel (9), whereas the polypeptide substrate transits through the pore ring of the SecY subunit (10). Retrospectively, the proposed mechanism of SecYEG channel gating is also supported by earlier studies. A specific class of mutations, called prl, into the SecY or SecE subunit allow the transport of proteins with defective or even missing leader peptides (11, 12). Biochemical studies showed that the mutation prlA4 enhances the translocation rates, abolishes the stimulatory effect of the PMF, and increases the affinity of the SecYEG complex for SecA (13, 14). Analyses of the stability of the SecYEG complexes carrying the prl mutations led to the proposal that these various phenotypes result from a loosened association among the SecYEG subunits (15). The crystal structure shows that most of the prl mutations are located in the pore ring or in the plug domain, and these mutations may indeed destabilize the closed state of the channel (6).

In the present study, we have analyzed further the contribution of the plug domain to the gating mechanism of the translocon. Our results show that the plug is not essential for the function of the SecYEG complex, both in vivo and in vitro. These results are in agreement with a recent study performed on the yeast translocon (16). However, in bacteria, we find that deletion of the plug enhances the activity of the channel during ATP-driven translocation and causes a phenotype similar to the Prl mutations. Accordingly, the absence of the plug results in loosened SecYEG subunit associations. The results suggest that the plug domain is not essential to seal the channel but...
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rather acts as a structural element necessary for the stability, regulation, and selectivity of the translocon.

EXPERIMENTAL PROCEDURES

Materials—ATP, AMP-PNP, carbonyl cyanide 3-chlorophenylhydrazone, and the monoclonal anti-hemagglutinin antibody were purchased from Sigma. β-octyl-glucoside and dodecyl-maltopyranoside (DDM) were purchased from Roche Applied Science and Anatrace. Mouse monoclonal antibody raised against the last cytoplasmic loop of SecY was a gift from Dr. I. Collinson. Polyclinidene fluoride membrane used in Western blotting was purchased from Bio-Rad. Goat antibody against the last cytoplasmic loop of SecY was a gift from Jackson Laboratories.

Plasmids and Constructs—The plasmid pBAD22-hisEYG and pBAD22-HAEGY carrying the prl mutations in used in this study were previously described (15, 17, 18). The structure of M. janinascii (Protein Data Bank: 1RH2) and the published sequence alignments (6) were used to identify the Escherichia coli aminoacyl residues forming the SecY plug domain. The two SecY plug deletions were performed by PCR amplification of the region on each side of the desired deletion followed by digestion and replacement into the parental vector pBAD22-HAEGY. Residues from position 62 to 69 (TM2a) and 42 to 74 (periplasmic loop P1) were deleted to form SecY8 and SecY33, respectively. The deleted regions are replaced by the Apal restriction site, which encodes for the sequence Gly-Pro. The residues at position Ser-68 and Ser-282 were replaced by cysteines in pBAD-hisEYG using the Transformer site-directed mutagenesis kit (Clontech). For plasmid expression of SecY in the absence of the SecE subunit, plasmid pBAD22-HAEGY was digested with NheI and SalI, treated with the Klenow fragment, ligated, and religated. The plasmid pCM10K-YsecY was built from pKY248 (19) and pCM10K (20). pCM10K-YsecY carries the origin of replication of pBR322, which is destabilized in strains containing the pcnB mutation, the rpsL+ gene, which confers dominant sensitivity to streptomycin, the secY+ gene under the control of the pLa promoter, and the resistance gene to kanamycin. Strain DB824 (MC4100, leu+araZ141, malE16, pcnB, secYaadA, recA::cat, rpsL150) was built in several steps. Briefly, the substitution of the open reading frame of secY by aadA was achieved in E. coli DY378 cells (21) carrying secY on the plasmid pKY248 (19). The product of aadA confers spectinomycin resistance, and the correct disruption of secY was checked by Southern blotting. Strain DB824, which carries secY+ on the plasmid pCM10K-YsecY+, was produced by P1-mediated transduction of the secY::aadA allele into E. coli DB757 followed by transduction of the recA deletion. The strain becomes resistant to 100 μg/ml spectinomycin because of the aadA gene but sensitive to 1.5 mg/ml streptomycin because of the presence of rpsL+. Plasmid pCM10K-YsecY is unstable in DB824 because of the pcnB mutation. The genes coding for SecY, PrlA3, and the two plug-less mutants proteins were cloned in pBAD104, an AmpR plasmid that contains the pSC101 origin of replication compatible with pCM10K-YsecY.

In Vitro and In Vivo Translocation Assays—Preparation of inner membrane vesicles (IMVs), SecA, proOmpA, and the procedure for 125I radiolabeling were previously described (17, 22). In vitro translocation assays were performed in 50 μl of TL buffer (50 mM Tris-HCl, pH 7.9; 50 mM NaCl; 50 mM KCl; 5 mM MgCl2) containing SecA (40 μg/ml), bovine serum albumin (200 μg/ml), IMVs (50 μg/ml), ATP (2 mM), and 125I-proOmpA (~60,000 cpm; 0.56 μg/ml; 15 nm). After an 8-min incubation at 37 °C, translocation reactions were stopped on ice, treated with proteinase K (1 mg/ml, 15 min), trichloroacetic acid-precipitated, and analyzed by 12% SDS-PAGE. Translocation efficiency was measured with a phosphorimaging device using the ImageQuant software and quantified by comparison with 125I-proOmpA standard curve. The SecA membrane insertion assay was performed using urea- stripped IMVs as described previously (23). The measure of alkaline phosphatase PhoA exported to the periplasm, and the biosynthetic labeling and immunoprecipitation of the maltose-binding protein MalE were performed using protocols described previously (24).

Intramolecular SecY Cross-links—Cross-link between the cysteine residues Cys-68 and Cys-282 was performed in 50 μl of TL buffer containing bovine serum albumin (200 μg/ml), IMVs (100 μg/ml), and the indicated amount of Cu2+(phenanthroline)3 (CP3) for 10 min at room temperature (9). Oxidized IMVs were layered over 1 volume of 0.2 m sucrose in TL buffer and resolated by ultracentrifugation (30 min, 4 °C, 55,000 rpm, Beckman TLA-55 rotor). IMVs were washed three times and resuspended in TL buffer with brief sonication. Cross-linking efficiency was monitored by trypsin digestion (30 min, 4 °C, 1 mg/ml trypsin).

RESULTS

Production of Plug-less Translocation Channels—The plug domain is located in the first periplasmic loop of SecY, just ahead of the transmembrane segment TM2. It is a short, distorted helix that seats atop of the channel, about half-way through the membrane (Fig. 1A). In most organisms, the plug region is connected to TM1 and TM2 by the sequences ΦFXG and ГΦXP, respectively (where Φ is a conserved hydrophobic amino acid). These motifs are proposed to serve as a hinge for the motion of the plug (6). In this study, we deleted the plug domain (residues 62–69; hereafter referred to as SecYΔ8) or most of the region delimited by the hinge motifs (residues 42–74; hereafter referred to as SecYΔ33) to minimize the possibility that the missing plug be replaced by adjacent residues from the loop.

To determine whether these two deletions affect the stability or activity of the SecY protein, the mutant genes were cloned in the plasmid pBAD22 and introduced into the strain DB703, which carries the secY24 mutation (25). secY24 encodes a SecY protein that is fully functional at 30 °C but sufficiently inactive at 42 °C to prevent colony formation on minimal medium glucose plates. The results show that partial complementation is obtained upon expression of the plasmids carrying the plug-less secY+ alleles (Fig. 1B, top panel), suggesting that activity of the plug-less translocons is somehow altered at 42 °C. Partial complementation is also observed when the plasmid expresses SecY containing the prlA3 mutation (mutation F67C in the plug domain).

To analyze further the functionality of the plug-less secY mutants, we tested the ability of the secY mutant alleles to com-
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FIGURE 1. Production of two plug-less translocation channels. A, a ribbon representation of SecYEG complex as seen from the periplasm. The residues deleted in SecYΔ33 (position 42–74) are shown in yellow. The helical plug, deleted in SecYΔ8 (residues 62–69), is colored in red. The residues Ser-68 (in the plug domain) and Ser-282 (in TM7) are marked with an asterisk. The SecE and SecG subunits are colored in blue and green, whereas the rest of the SecY subunit is in blue. B, top panel, overnight cultures of DB703 (MC4100, leu::Tn10, secY24) containing the plasmid encoding for the indicated SecY protein were washed twice in M63 medium. Serial dilutions were spotted onto LB agar plates containing ampicillin (100 μg/ml) and streptomycin (1.5 mg/ml) and incubated overnight at 37 °C. Bottom panel, overnight cultures of DB824 (see “Experimental Procedures”) containing the plasmid encoding the indicated SecY protein were washed twice in M63 containing 0.2% arabinose and incubated at 42 °C. C, SecY, SecE, and SecG subunits are colored in blue, green, whereas the rest of the SecY subunit is in blue. A, top panel, overnight cultures of DB703 (MC4100, leu::Tn10, secY24) containing the plasmid encoding for the indicated SecY protein were washed twice in M63 medium. Serial dilutions were spotted onto LB agar plates containing ampicillin (100 μg/ml) and streptomycin (1.5 mg/ml) and incubated overnight at 37 °C. B, top panel, overnight cultures of DB703 (MC4100, leu::Tn10, secY24) containing the plasmid encoding for the indicated SecY protein were washed twice in M63 medium. Serial dilutions were spotted onto LB agar plates containing ampicillin (100 μg/ml) and streptomycin (1.5 mg/ml) and incubated overnight at 37 °C. Bottom panel, overnight cultures of DB824 (see “Experimental Procedures”) containing the plasmid encoding the indicated SecY protein were washed twice in M63 containing 0.2% arabinose and incubated at 42 °C.

M63 medium. Serial dilutions were spotted onto LB agar plates containing ampicillin (100 μg/ml) and streptomycin (1.5 mg/ml) and incubated overnight at 37 °C. Overproduction of the SecYEG mutants complexes in strain BL21. IMVs proteins (1 μg of proteins) were separated by 12% SDS-PAGE, transferred onto polyvinylidene difluoride, and immunostained with the indicated antibodies.
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A

\[
\begin{align*}
\text{PMF} & \quad \text{pOA} \\
+50 & \quad 12.0 \\
-50 & \quad 0.9 \\
\text{OA} & \quad 0.8 \\
\ \text{pmol/min/mg} & \quad \text{pmol/min/mg} \\
Y & \quad 5.5 \\
Y^{Δ8} & \quad 7.5 \\
Y^{A33} & \quad 8.0 \\
Y^{A3} & \quad 6.3 \\
Y^{A4} & \quad 17.9 \\
\end{align*}
\]

B

\[
\begin{align*}
\text{pOA} & \quad \text{μM} \\
0 & \quad 0.2 \\
0.2 & \quad 0.4 \\
0.4 & \quad 0.6 \\
0.6 & \quad 0.8 \\
0.8 & \quad 1.0 \\
\text{pmol/min/mg} & \quad \text{pmol/min/mg} \\
Y & \quad 0 \\
Y^{Δ8} & \quad 50 \\
Y^{A33} & \quad 50 \\
Y^{A3} & \quad 50 \\
Y^{A4} & \quad 50 \\
\end{align*}
\]

C

\[
\begin{align*}
\text{Y} & \quad \text{SecA} \\
\text{Y}^{Δ8} & \quad \text{30 kDa} \\
\text{Y}^{A33} & \quad \text{OA} \\
\text{Y}^{A3} & \quad \text{OA} \\
\text{Y}^{A4} & \quad \text{OA} \\
\end{align*}
\]

FIGURE 2. In vitro activity of the plug-less translocation channel. A, translocation of \(^{125}\text{I}\)-labeled proOmpA (pOA) (60,000 cpm, 28 ng) into IMVs enriched for the indicated SecYEG complex in the presence or absence of PMF. OA, OmpA, B, quantitative analysis of proOmpA translocation into the same set of IMVs as in A: SecY\(\,\), SecYA3\(\,\), SecY\(Δ8\),\(\,\), and SecY\,A33\(\,\). ProOMP A quantity ranged from 0.04 to 1.6 \(μ\)g per assay (0.02–0.86 \(μ\)g). The PMF was dissipated using carbonyl cyanide 3-chloro-phenylhydrazone (20 \(μ\)M). proOmpA translocation reached ~200 pmol/min/mg with the SecYEG complex carrying the prlA4 mutation. The resulting curve was omitted from the graph to help appreciate the results obtained with the other complexes. C, SecA membrane insertion was measured as described previously (Economidou and Wickner (23)) using urea-stripped IMVs. The reaction was initiated in the presence of proOmpA (0.53 \(μ\)M) and ATP (1 \(μ\)M) for 10 min at 37 °C followed by the addition of AMP-PNP (4 \(μ\)M) for 3 min.

Next, we tested the ability of the plug-less translocation channel to generate the “membrane-inserted state” of SecA, as assessed by the formation of a SecA-30-kDa protease-protected domain (23). The SecA membrane insertion occurs at the initial step of the translocation cycle, and it represents another way to monitor the activity of the translocon. The results show that the prlA3 and prlA4 mutations increase the yield of protease-protected SecA, in agreement with previous studies (14, 15). Similarly, the two plug-less mutants increase the formation of the SecA-30-kDa protease-protected domain (Fig. 2C).

Since the plug-less and PrlA mutant complexes present similar in vitro translocation activities, we tested whether the deletion of the plug also confers a Prl phenotype in vivo. The Prl phenotype is characterized by the capacity of the cells to export proteins with a defective leader peptide (28). The plasmids encoding the SecYEG mutant complexes were transformed into a strain producing PhoA\((Δ8–9)\) (29), and the amount of exported alkaline phosphatase was measured in the periplasmic fraction of the cells (Fig. 3A). The results show that the deletion of the plug effectively suppresses the translocation defect of PhoA\((Δ8–9)\). The suppression obtained with the SecY\(Δ8\) mutant complex is almost as strong as that observed with the complex carrying the prlA3 or prlA4 mutation. As a complementary assay, we measured the export efficiency of the maltose-binding protein MalE(T16K) (30) using radioactive pulse labeling followed by immunoprecipitation and densitometry scanning. As determined by the extent of signal sequence cleavage, only 10% of the MalE(T16K) protein is exported during the radioactive pulse in cells expressing the wild-type SecYEG complex (Fig. 3B). In contrast, in cells expressing the plug-less SecYEG complexes, the export is strongly improved and almost reaches the level observed in cells expressing the PrlA3 mutant complex. Altogether, the results show that the deletion of the plug domain increases the activity of the SecYEG channel but reduces its selectivity.

Structural Stability of the Plug-less Translocation Channel—When extracted from the lipid bilayer with detergents, the SecYEG complex easily dissociates into single subunits (31). This property was exploited to demonstrate that a fundamental distinction between the wild-type and the PrlA mutant channel resides in the strength of the SecYEG subunit associations (15). The two plug-less SecYEG complexes were thus tested for the stability of their quaternary structure upon extraction from the membrane with DDM. The solubilized membrane proteins

FIGURE 3. In vivo activity of the plug-less translocation channel. A, strain Mph53 (MC4100, prlA82, Δ[Leu8-Ala9]) transformed with plasmids encoding for the indicated SecYEG mutant complex was grown at 37 °C in LB medium until an OD\(_{600}\) = 0.5 and then induced with 0.2% arabinose for 60 min. Alkaline phosphatase (AP) activity was measured as described previously (Bost and Belin (24)). Each bar represents the average of three independent cultures. B, strain DB757 (MC4100, araА714, malE16, pcnB) transformed with plasmids encoding for the indicated SecYEG mutant complex was induced for 60 min with 0.2% arabinose and for 20 min with 0.2% maltose. After \(^{35}\)S-methionine pulse labeling (30 s), the maltose-binding protein MalE was immunoprecipitated from the cell lysates. The amount of precursor and mature MalE was measured, and the relative amount of mature MalE is plotted on a graph. Each bar represents the average of two independent experiments.
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were analyzed by blue native-PAGE (BN-PAGE (32)) followed by immunostaining with SecG antibodies (Fig. 4A). As reported previously (22), the SecYEG complex migrates as a population of monomers and dimers, and the same behavior is observed for the SecYEG complex carrying the prlA3 or prlA4 mutation. In contrast, signs of complex instability are observed when analyzing the SecYEG complex with the large SecYΔ33 deletion. With this mutant, the population of SecYEG monomer-dimer is strongly reduced with a concomitant increase of dissociated products, migrating below the SecYEG monomer. The SecYΔ8 deletion also appears to destabilize the SecYEG channel but to a lesser extent.

To analyze further the strength of the SecYEG association, the plug-less and PrlA4 mutant complexes were His-tagged and purified by Ni²⁺-chelating chromatography in the presence of DDM, as described previously (18). With SecYΔ33 deletion, the purification yielded only the SecE subunit, as expected since the SecYEG complex readily dissociates during incubation with the detergent (data not shown but see Fig. 4A). The SecYEG complex carrying the secYΔ8 deletion was, however, sufficiently stable to allow its purification, and it was obtained at a yield comparable with the wild-type or the PrlA4 complex. Next, these purified complexes were incubated at room temperature with β-octyl-glucoside and then analyzed by BN-PAGE (Fig. 4B). The detergent β-octyl-glucoside was used in the past to monitor the strength of the SecYEG subunit association (15, 31). The results show that the oligomeric state of the SecYEG complex is shifted toward the monomer when it carries the prlA4 or secYΔ8 mutation (Fig. 4B, left panel). Furthermore, incubation with a higher amount of detergent dissolves further the PrlA4 and SecYΔ8 mutant complexes into single subunits, whereas the wild-type complex remains mostly intact in these conditions (Fig. 4B, middle panel). Altogether, the results show that the SecYEG subunit associations are more fragile when the SecY plug is absent or when SecY carries the prlA4 mutation. The results agree with the proposal that the stability of the SecYEG structure is correlated to the activity of the channel (15).

The Dynamics of the Plug Are Essential for Protein Translocation—Disulfide cross-linking studies have shown that the plug domain moves away from the center of the channel during protein translocation (9), but this study reveals that the plug domain is not essential per se for the function of the translocon. We thus asked whether the plug dynamics are essential for the translocation reaction. This was achieved by reducing the mobility of the plug by cysteine cross-linking. The crystal structure of the M. jannaschii SecY complex allowed us to predict that the E. coli SecY residues Ser-68 (located in the plug domain) and Ser-282 (located in TM7) would be close to each other in the resting (closed) state of the channel. These 2 aminoacyl residues were replaced by cysteine residues, and the resulting SecYEG complex was overproduced in E. coli. The membranes were isolated and oxidized with CP₃ before being resolated. The presence of a trypsin cleavage site located in the cytosolic loop between TM6 and TM7 of SecY (33) allowed monitoring the efficiency of disulfide linkage between the residues Cys-68 and Cys-282 (Fig. 5A). In the presence of trypsin, the SecY protein is digested into two fragments, and the C-terminal fragment (∼14 kDa) is detected using an antibody recognizing the most C-terminal cytoplasmic loop of SecY (Fig. 5A, top panel). When the residues Cys-68 and Cys-282 form a disulfide bond, the N- and C-terminal fragments remain tethered together, and the trypsinized-SecY migrates like the uncleaved SecY on non-reducing SDS-PAGE (Fig. 5A, lower panel). This disulfide bond depends on the presence of both cysteines in SecY (data not shown), and the results show that an efficient covalent linkage is obtained upon incubation of the IMVs with 200 μM CP₃ (Fig. 5A).

The membranes containing the oxidized SecYEG complex were then tested for their protein translocation activity (Fig. 5B). The results show that the progressive oxidation of the SecYEG complex leads to a concomitant decrease of the protein translocation efficiency. At 200 μM CP₃, the plug domain is covalently linked to residue Cys-282 of TM7 (Fig. 5A), and the translocation activity is reduced to a level comparable with that
of wild-type membranes \(i.e\). containing only chromosomally encoded wild-type SecYEG. This reduction of translocation activity depends on the presence of both Cys-68 and Cys-282 in SecY (Fig. 5B). Thus, the mobility of the plug is essential to the mechanism of protein translocation.

**DISCUSSION**

The crystal structure of the SecY complex represents the closed state of the channel because the central passage is blocked by the plug domain and the constriction ring. For the channel to open, the plug needs to move away, and the constriction ring must expand to widen the passage for outgoing polypeptide substrate. Computer-based simulations support this model (34–36), and experimental data show that the plug domain is indeed relocated outside the channel toward the C terminus of the SecE subunit during translocation (9, 37). Furthermore, it has been shown that locking the plug in the open state enhances the in vitro activity of the translocon (9), whereas locking the plug in its closed position (this study) abolishes translocation. These experimental observations clearly indicate that the movement of the plug is an essential subreaction occurring during polypeptide transport across the membrane.

The function of the plug is, however, not yet fully understood. Earlier \(in\) vivo experiments showed that the combination of the mutations \(prlA3\) (F67C in the plug domain of SecY) with a cysteine at position 120 in SecE (periplasmic end of SecE) results in synthetic lethality (37). Because formation of a disulfide bridge between the 2 cysteines should leave the channel in a permanently open state, it was logical to suppose that an important function of the plug is to close the channel pore to small molecules. However, both this study in bacteria and a recent study in yeast (16) rather indicate that the plug domain is not essential for cell viability or for the general function of the translocon. A partial growth defect is observed on minimal medium at 42 \(^\circ\)C when the plug-less SecY protein is overproduced in the absence of its interacting partner SecE (Fig. 1B). The reason remains unclear and could be linked to the stress of cells growing on minimal medium (38). In yeast, the absence of the plug reduces the steady state level and the activity of the translocon, but the cells are viable (16). Here, we show that overproduction of the plug-less SecYEG complex is possible and does not yield dominant-negative effects. This last observation clearly indicates that the plug domain is not required per se to seal the channel in the resting state. Instead, the constriction ring may be sufficient to hinder ions and other molecules from permeating through the channel. In eukaryotes, there are evidences that the lumenal chaperone BiP forms an alternative or additional sealing system on the extracytoplasmic side of the translocon (39, 40).

Although the role of the plug domain as a membrane seal before or during protein transport cannot be ruled out, our results suggest another function. The results show that the deletion of this domain converts the SecYEG complex into a “Prl-like” translocon. In the majority, the \(prl\) in \(secY\) mutations are confined to the pore and plug domains (6, 41), and they confer various \(in\) vivo and \(in\) vitro phenotypes: enhanced translocation rates, increased membrane affinity and insertion of SecA, diminished requirement for canonical leader peptides, reduced PMF dependence of translocation, facilitated translocation of folded domains, and for some leader peptides or TM segments, inversion of their membrane topology (13, 14, 42, 43). It was proposed that these seemingly unrelated effects all derive from an enhanced conformational flexibility of the translocation channel (15). Accordingly, the SecYEG complex is expected to be very dynamic to let the polypeptide substrate move through or be released laterally into the lipid bilayer. Our results show that the plug domain interacts with the middle part of TM7 (forming part of the lateral gate), whereas the deletion of the plug domain weakens the SecYEG associations. It is thus possible that the plug domain in the resting position interacts with the inner walls of the channel, which, in turn, contributes to stiffen and stabilize the structure of the channel in the closed state. In such a scenario, binding of the leader peptide between TM2 and TM7 (8) would disrupt the interaction between the plug domain and the walls of the channel, provoke the relaxation of the channel structure, and enable the widening of the central pore to open the channel for translocation.
In conclusion, the genetic selections yielded prl mutations that enhance the dynamics of the plug domain and loosen the SecYEG association of the subunits, whereas preserving sufficient stability for the channel to avoid dissociation. More radical mutations such as the deletion of the plug domain do not affect the basic function of the translocon but, like the prl mutations, relax the channel structure and deregulate its activity. In the absence of the plug domain, the channel becomes less sensitive to the stimulatory effect of the PMF and less selective toward the composition of the leader peptide.

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