Involvement of SecDF and YidC in the Membrane Insertion of M13 Procoat Mutants †

ARTICLE *in* BIOCHEMISTRY · SEPTEMBER 2005

Impact Factor: 3.02 · DOI: 10.1021/bi047418k · Source: PubMed

Involvement of SecDF and YidC in the Membrane Insertion of M13 Procoat Mutants†

Minyong Chen,‡ Kun Xie,‡ Jijun Yuan,‡ Liang Yi,‡ Sandra J. Facey,§ Nathalie Pradel,|| Long-Fei Wu,|| Andreas Kuhn,§ and Ross E. Dalbey*,‡

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, Institute of Microbiology and Molecular Biology, University of Hohenheim, Garbenstrasse 30, 70599 Stuttgart, Germany, and Laboratoire de Chimie Bacterienne, UPR9043, Institut de Biologie Structurale et Microbiologie, CNRS, 31, chemin Joseph Aiguier, 13402 Marseille Cedex 20, France

Received December 8, 2004; Revised Manuscript Received April 21, 2005

ABSTRACT: The M13 phage Procoat protein is one of the best characterized substrates for the novel YidC pathway. It inserts into the membrane independent of the SecYEG complex but requires the 60 kDa YidC protein. Mutant Procoat proteins with alterations in the periplasmic region had been found to require SecYEG and YidC. In this report, we show that the membrane insertion of these mutants also strongly depends on SecDF that bridges SecYEG to YidC. In a cold-sensitive mutant of YidC, the Sec-dependent function of YidC is strongly impaired. We find that specifically the SecDF-dependent mutants are inhibited in the cold-sensitive YidC strain. Finally, we find that subtle changes in the periplasmic loop such as the number and location of negatively charged residues and the length of the periplasmic loop can make the Procoat strictly Sec-dependent. In addition, we successfully converted Sec-independent Pf3 coat into a Sec-dependent protein by changing the location of a negatively charged residue in the periplasmic tail. Protease mapping of Pf3 coat shows that the insertion-arrested proteins that accumulate in the YidC- or in the SecDF-deficient strains are not translocated. Taken together, the data suggest that the Sec-dependent mutants insert at the interface of YidC and the translocon with SecDF assisting in the translocation step *in vivo*.

The YidC pathway is a newly defined membrane insertion pathway in bacteria. It is used for the insertion of the M13 Procoat protein (*1*−*3*) and Pf3 phage protein (*4*) as well as for assembly of some respiratory substrates such as subunit c of the F_1F_0 ATP synthase (*5*−*7*). While YidC is required for membrane insertion of a subset of proteins, it does not generally play a critical role for protein export (*1*, *8*). Interestingly, YidC alone is sufficient for the insertion of the Pf3 coat protein (*9*) and subunit c of F_1F_0 ATP synthase (*10*). With these protein substrates, it functions as an insertase, promoting their insertion into the membrane. In mitochondria and chloroplasts, there is a homologous membrane insertion pathway involving Oxa1 and Alb3 (*11*−*14*).

Most of the tested membrane proteins are inserted into the lipid bilayer using the Sec translocase. These include FtsQ (*15*, *16*), leader peptidase (*17*, *18*), mannitol permease (*19*), YidC (*20*, *21*), MalF (*22*, *23*), and Lac permease (*24*). The Sec translocase is comprised of the SecYEG and SecDFYajC heterotrimeric complexes. The SecYE proteins form the core of the protein-conducting channel (*25*) and are essential for protein translocation (*26*). The 3.2 Å resolution structure of the archaeal Sec61$\alpha\beta\gamma$ (SecYEG) complex shows that the membrane-embedded protein-conducting channel is comprised exclusively of the Sec61α polypeptide, which corresponds to SecY in *Escherichia coli* (*27*). While SecDF does not contribute to the structure of the channel, it nevertheless contributes to the translocation process (*28*, *29*).

Recently, it has been discovered that a portion of YidC cofractionates with the SecYEGDFYajC complex during purification (*30*). Specifically, YidC forms a complex with SecDFYajC, and SecDFYajC functions as a bridge to link YidC to the SecYEG machinery (*31*). SecDF is believed to play a minor role in the export of proteins (*28*, *29*), although it has not been thoroughly investigated for insertion of membrane proteins. The requirement of YidC for protein translocation in the Sec pathway is generally not so strict, in contrast to the role YidC has in the Sec-independent pathway. For instance, depletion of YidC only has a small inhibitory effect on the membrane insertion of the Sec-dependent leader peptidase (*1*) and FtsQ (*15*). Insertion of Sec-dependent leader peptidase is also inhibited at the nonpermissive temperature in a cold-sensitive (cs) YidC mutant (*32*). However, the cs YidC mutant can efficiently mediate the insertion of wild-type Procoat at the nonpermissive temperature. Therefore, it appears that the Sec-related YidC function may be highly impaired in this conditional lethal mutant. For Sec-dependent proteins, YidC may func-

† The study is supported by NIH Grant to R.E.D. (GM63862), DFG Grant (Ku749/3-2) to A.K., and a grant from Programme de Recherches Avancées (from AFCRST, PRA B99-03) to L.-F.W.

* To whom correspondence should be addressed: Department of Chemistry, The Ohio State University, 100 W. 18th Ave., Columbus, OH 43210. E-mail: dalbey@chemistry.ohio-state.edu. Telephone: (614) 292-2384. Fax: (614) 292-1532.

‡ The Ohio State University.

§ University of Hohenheim.

|| CNRS.

10.1021/bi047418k CCC: $30.25
Published on Web 07/12/2005

tion to move hydrophobic domains out of the SecYEG channel into the lipid bilayer (*15*) and/or assists in the folding of the hydrophobic domains of integral membrane proteins (*14*, *33*).

It is puzzling how hydrophilic domains of membrane proteins cross the apolar membrane by the YidC pathway. YidC on its own may have a limited translocation ability since its substrates, wild-type Procoat and Pf3 coat, which have short translocated regions, can insert into the membrane in a manner independent of Sec machinery. In contrast, two Procoat mutants with additional negatively charged residues required SecA and SecY for efficient insertion (*34*). These include the −3M Procoat with three negatively charged residues in the center of the periplasmic loop and the −5 Procoat with five negatively charged residues in the amino-terminal region of the loop. Since these two mutants are still strictly YidC-dependent, it is possible that the translocation of their periplasmic domain is not ideal for YidC working on its own. For these mutants, YidC may require the assistance of Sec machinery. Therefore, the studies of the two Procoat mutants may provide a starting point for investigating the Sec-related function of YidC and elucidate the features which determine when the Sec components are necessary for translocation.

In this paper, we show that the membrane insertion of these Procoat mutants also strongly depends on SecDF that links SecYEG to YidC. In a cold-sensitive mutant of YidC, the Sec-related function of YidC is strongly impaired. We find that specifically the insertion of the SecDF-dependent mutants is strongly impaired in the cold-sensitive YidC strain. We find also that subtle changes in the periplasmic loop can make the Procoat proteins strictly Sec-dependent. In addition, we converted a Sec-independent Pf3 coat into a Sec-dependent protein by moving a negatively charged residue to a different position in the periplasmic region. Protease mapping of Pf3 coat shows that the insertion-arrested proteins that accumulate in the YidC or in the SecDF deficient strains are not translocated. The combined data are consistent with the Sec-dependent mutants inserting at the interface of YidC and the translocon with SecDF facilitating the translocation event.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, and Growth Conditions. E. coli YidC depletion strain JS7131 (*1*) and YidC cold-sensitive strain MYC-cs (*32*) are from this laboratory. SecE depletion strain CM124 was obtained from C. Murphy. SecDFYajC depletion strain JP325 (*28*, *35*) was obtained from J. Beckwith. JP325 is a RecA$^+$ version of the SecDF depletion strain, JP352, which was reported previously (*35*). Construction of several of the Procoat derivatives (PClep) and the Procoat 828 constructs have been reported (*2*, *34*, *36*−*38*). To express PClep in the JP325 or JS7131 strains, PClep constructs were cloned into the pMS119 vector, which contains the IPTG-inducible *tac* promoter and the *lacI*^{q}. To express PClep in the CM124 strain, the PClep genes were cloned into the pEH1 vector (*39*) under the control of the *lacUV5* promoter. pET606 (*31*), which contains SecDFYajC genes under control of the *tac* promoter, was obtained from A. Driessen. Plasmid p8792, which contained the etpM49−cvaC gene fusion protein (*40*), was from L.-F.W.'s laboratory. Trans ^{35}S label, a mixture of 85% [^{35}S]methionine and 15% [^{35}S]-cysteine (1000 Ci/mmol), was from ICN.

To deplete SecDF within the cell, an overnight culture of JP325 strain (*ara*Δ714 Δ[*argF-lac*]U169 *rps*L150 *rel*A1 *thi flb*5306 *deo*C1 *pts*F25 *tgt*::*kan ara*C$^+$ P$_{BAD}$::*yajCsecDF*) bearing the PClep or Pf3 coat mutants was back-diluted 1:100 in LB supplemented with glucose (0.2%), and the cells were grown to an A_{600} of ∼0.7. The culture was then back-diluted again 1:100 in LB medium containing glucose (0.2%) or in LB medium containing arabinose (0.2%), and the cells were grown until the A_{600} of the arabinose culture is ∼0.2 unit more than the A_{600} of glucose culture. The cells were then pelleted and resuspended in M9 minimal medium (*41*), and the culture was incubated at 37 °C for 30 min. The cultures were then subjected to radiolabeling with [^{35}S]methionine (see the next section). For the SecE depletion studies, CM124-bearing PClep mutants were grown and analyzed as previously described (*42*). To deplete YidC within the cell, JS7131-bearing PClep or Pf3 coat mutants were grown as described previously (*4*). For inactivation of the cs YidC, the *E. coli* MYC-cs cells bearing the PClep and PC828 mutants were grown as described previously (*32*). Briefly, the MYC-cs cells were grown in LB medium to an A_{600} of ∼0.4 at the permissive temperature (37 °C), pelleted, resuspended in M9 minimal medium, and incubated at 37 °C for 30 min. Half of the culture was then shifted to the nonpermissive temperature (25 °C), and the other half remained at the permissive temperature (37 °C). The cultures were then grown for 2 h, and then subjected to radiolabeling with [^{35}S]methionine (see section immediately below).

Signal Peptidase Processing Assay and Proteinase K Protection Assay. Prior to labeling, 1 mM IPTG was added to the cultures for 5 min to induce the expression of the plasmid-encoded membrane proteins. The cells were then labeled with *trans*-[^{35}S]methionine (100 μCi/mL of cells) for 20 s, and chased with nonradioactive methionine for the indicated times. To analyze PClep, PC828, and PC828ΔH2, the labeled cells were precipitated with trichloroacetic acid (TCA). For analysis of Pf3 coat, radiolabeled cultures were converted to spheroplasts and incubated in the presence or absence of proteinase K (final concentration of 0.5 mg/mL) for 60 min on ice. The samples were then precipitated with TCA. Pf3 coat and PClep expressed in the JP325 strain or in JS7131 were detected directly without immunoprecipitation (*4*, *32*). Leader peptidase (Lep)[1] antiserum was used to immunoprecipitate PClep expressed in the CM124 strain. Procoat and OmpA antisera were used to immunoprecipitate PC828 and PC828ΔH2 in the JP325 strain, respectively (*2*, *36*).

PspA Immunodetection. The MYC-cs mutant and its isogenic parental strain (MC1060) carrying the plasmid p8792 were incubated at the permissive temperature (37 °C) in the presence of glucose until the A_{600} reached 0.1. Aliquots of the cultures were shifted to 24 °C, while the control cultures were kept at 37 °C. At an A_{600} of 0.2, the growth medium of one set of cultures was replaced with fresh LB supplemented with arabinose and another set with fresh LB and glucose and grown for an additional 30 min. In this

[1] Abbreviations: OmpA, outer membrane protein A; lep, leader peptidase; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

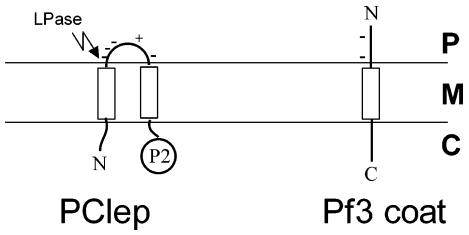

FIGURE 1: Membrane topology of Procoat Lep (PClep) and Pf3 coat. Apolar domains are represented by rectangles, and the leader peptidase (LPase) cleaves Procoat on the periplasmic side of the membrane. Proteinase K accessibility in spheroplasts is used to monitor the translocation of the periplasmic region of Pf3 coat. The charges within the periplasmic domains of PClep and Pf3 coat are indicated. The P2 domain consists of 103 residues from the periplasmic domain of leader peptidase. P is the periplasm, M the membrane, and C the cytosol.

study, arabinose was used to induce the etpM−colicin V chimera from plasmid p8792 which is under the control of the *araBAD* promoter.

The cells were lysed with SDS loading buffer, and the cell lysates were resolved by electrophoresis on polyacrylamide gels (12.5%) in the presence of sodium dodecyl sulfate (SDS, 0.1%), immobilized onto polyvinylidene difluoride (PVDF) membranes, and analyzed by immunoblot using the ECL kit according to the manufacturer's instructions (Amersham Biosciences). Polyclonal antibodies against PspA were used at a 1:2000 dilution in the immunoblot.

RESULTS

SecDF Is Required for the Insertion of a Subset of Procoat Mutants. SecDF has been shown to facilitate the export of proteins across the membrane (*28*, *35*). In addition, it has also been reported that SecDF interacts with YidC, and this interaction links YidC and Sec machinery (*31*). However, whether SecDF plays a role in membrane protein insertion has not been thoroughly investigated. M13 Procoat is a Sec-independent and YidC-dependent protein. We have found the addition of a leader peptidase (Lep) soluble domain P2 (PClep) does not affect M13 Procoat membrane insertion; i.e., wild-type (wt) PClep is still strictly Sec-independent and YidC-dependent. −4PClep, −5PClep, and −3MPClep are PClep mutants with negatively charged residues introduced into the periplasmic loop (see Figures 1 and 2A). −4PClep, −5PClep, and −3MPClep are still strictly YidC-dependent (*2*); however, the insertion of −5PClep and −3MPClep is not as efficient as wt PClep and −4PClep in a SecY temperature-sensitive mutant at the nonpermissive temperature (*34*).

Therefore, we used these PClep mutants to investigate whether SecDF plays a role in membrane protein insertion, as well as to study the relationship between YidC and Sec machinery. JP325, which has SecDFYajC under the control of the araBAD promoter, was grown under SecDF depletion conditions (glucose) or SecDF-expressed conditions (arabinose). Figure 2B shows that the insertion of wt PClep is independent of the Sec machinery; i.e., wt PClep was rapidly processed to mature protein when grown under normal and limiting SecDF conditions. Processing is also quite rapid for the −4PClep proteins when SecDF is depleted (Figure 2B, Glc). In contrast, the depletion of SecDF leads to a complete block in membrane insertion of the Sec-dependent −5PClep

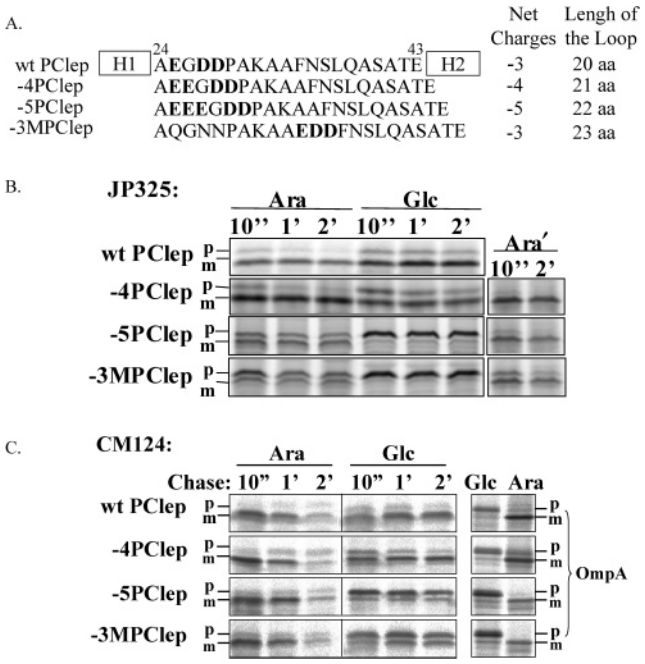

FIGURE 2: SecDF and SecE are required for the insertion of −5PClep and −3MPClep. (A) The amino acid sequences of the periplasmic domain in wild-type (wt) PClep, −4PClep, −5PClep, and −3MPClep are depicted. The net negative charges (in bold) and the length of the periplasmic loop of each mutant are indicated. The positions of the first (A24) and last (E43) amino acid residues in the periplasmic domain of wt PClep are indicated. aa stands for the amino acid residues, and H1 and H2 denote the hydrophobic domains 1 and 2 of Procoat, respectively. H1 is part of the cleavable leader sequence, and H2 constitutes the membrane anchor domain in the mature domain of Procoat. (B) The insertions of wt PClep, −4PClep, −5PClep, and −3MPClep were tested in the JP325 strain. JP325 cells bearing the wt PClep or PClep mutants were back-diluted (1:100) to LB medium containing glucose, and grown to an A_{600} of ∼0.7. The cultures were then back-diluted (1:100) again to LB medium containing arabinose (Ara) and glucose (Glc). After induction with IPTG for 5 min and labeling with [^{35}S]methionine, the cells were chased with nonradioactive methionine for 10 s, 1 min, and 2 min. In the right panel, JP325 cells bearing −4PClep, −5PClep, and −3MPClep were continuously grown under arabinose conditions (Ara′). They did not undergo the back-dilution to LB containing glucose as described for the left panel (Ara). (C) The Sec dependency of membrane insertion was analyzed using the SecE depletion strain. CM124 cells bearing wt PClep, −4PClep, −5PClep, and −3MPClep were grown in arabinose (Ara, SecE present) and glucose (Glc, SecE depleted) conditions. After the CM124 cells were induced with IPTG, labeled with [^{35}S]methionine, and chased with cold methionine, PClep and OmpA in the cells were immunoprecipitated and analyzed by SDS−PAGE and phosporimaging. In panels B and C, p and m denote the precursor form and mature form, respectively.

and −3MPClep (Figure 2B). The insertion block is much more severe than the block previously observed in the SecY and SecA temperature-sensitive strains (*34*). We also notice that −5PClep and −3MPClep do not insert into the membrane efficiently under the arabinose condition (Figure 2B, Ara). This is due to the fact that we deplete SecDF in glucose for several generations before the next back-dilution to the LB medium containing arabinose or glucose (see Experimental Procedures). When we grew the cells continuously in LB medium supplemented with arabinose (without the first depletion in glucose condition), −4PClep, −5PClep, and −3MPClep can insert into the membrane efficiently (Figure 2B, Ara′). These data suggest that SecDF plays an important role in the insertion of a subset of proteins such

as −5PClep and −3MPClep. It also indicates that −5PClep and −3MPClep are strictly Sec-dependent proteins.

To further confirm the Sec dependency of the PClep mutants, we also used the SecE depletion strain, CM124. Figure 2C shows that wt PClep and −4PClep can efficiently insert into the membrane when SecE is present (Ara) or depleted (Glc), confirming they are Sec-independent proteins. To confirm that SecE was depleted under glucose conditions, we examined the export of ProOmpA, which is Sec-dependent (Figure 2C, right panel). In the presence of glucose, ProOmpA accumulated in the cell, indicating that SecE was depleted. The insertion of −5PClep and −3M-PClep was strongly inhibited when SecE was depleted (Figure 2C, Glc). Most of −5PClep (∼70%) and ∼50% −3MPClep remained in a precursor form (p) after a 2 min chase when SecE was depleted (Figure 2C, Glc). These data confirm that −5PClep and −3MPClep are Sec-dependent.

The Insertion of Sec-Dependent Procoat Mutants Is Severely Blocked in the YidC Cold-Sensitive (cs) Strain, MYC-cs. Previously, we have isolated a YidC cold-sensitive (cs) mutant, and found that the Sec-independent wt PClep can still insert efficiently into the membrane while Sec-dependent Lep insertion is impaired in the YidC cs mutant at the nonpermissive temperature (*32*). This suggests that the Sec-related function of YidC may be impaired in the YidC cs mutants. To test this idea, we used the Sec-dependent PClep mutants to systematically determine the features that make a YidC-dependent protein require the Sec-related function of YidC. wt PClep, −4PClep, −5PClep, and −3MPClep were introduced into the YidC cs strain, MYC-cs. After the cells had grown at the permissive (37 °C) or nonpermissive (25 °C) temperature, MYC-cs cells expressing the PClep mutants were pulse-labeled with [^{35}S]methionine for 20 s and chased with nonradioactive methionine for the indicated times. Samples were then analyzed using SDS−PAGE and phosphorimaging. Figure 3A shows that wt PClep can efficiently insert into the membrane in the YidC cs strain, MYC-cs, at 37 and 25 °C as reported previously (*32*). There is only a very small kinetic effect at the nonpermissive temperature in the YidC cs strain; 80% of PC-lep can insert at the nonpermissive temperature at the early chase point. In contrast, −4PClep, −5PClep, and −3MPClep, which have small changes in the periplasmic loop, are severely blocked in MYC-cs at 25 °C (Figure 3A). Strikingly, the insertion of −5PClep and −3MPClep was strongly inhibited even at the permissive temperature (37 °C) (Figure 3A). These two mutants can efficiently insert into the membrane with wild-type YidC at 37 °C (Figure 3A, right panel). As a control, we confirm these mutants can efficiently insert into the membrane within a 10 s chase at both 25 and 37 °C in the wild-type strain, MC1060 (Figure 3A, right panel).

We also tested whether the insertion/translocation of two additional Sec-dependent Procoat mutants, Procoat-828 (PC828) and PC828ΔH2, is also blocked in the YidC cs strain at the nonpermissive temperature. PC828 is a Procoat mutant in which the periplasmic loop was lengthened by a 174-amino acid OmpA fragment (*36*), and PC828ΔH2 is the PC828 mutant with a deletion of the Procoat membrane anchor (H2 domain) (*2*, *37*). These two mutants require the Sec complex for efficient insertion or translocation (*36*, *37*). Figure 3B shows that most of the PC828 protein is still in the precursor form and cannot insert into the membrane even

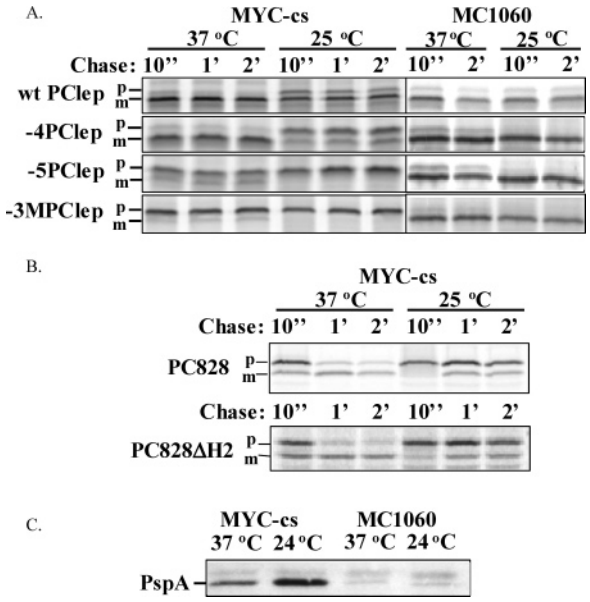

FIGURE 3: Insertion of Sec-dependent PClep is severely blocked in the YidC cold-sensitive (cs) strain, MYC-cs. (A) MYC-cs or MC1060 cells bearing wt PClep, 4PClep, −5PClep, and −3MPClep were grown at 25 or 37 °C, as described in Experimental Procedures. After induction with IPTG for 5 min, the cells were labeled with [^{35}S]methionine for 20 s and chased with nonradioactive methionine for 10 s, 1 min, and 2 min. The cells were then TCA precipitated and analyzed by SDS−PAGE and phosphorimaging. (B) MYC-cs cells bearing PC828 and PC828ΔH2 were grown as described for panel B. After labeling with [^{35}S]methionine had been carried out and a chase with cold methionine for the indicated times, PC828 and PC828ΔH2 were immunoprecipitated and analyzed by SDS−PAGE and phosporimaging. In panels A and B, p and m denote the precursor form and mature form, respectively. (C) Expression of the stress protein PspA is increased in the MYC-cs strain. Crude extracts (10 μg of protein each) were prepared from MYC-cs/p8792 or MC1060/p8792 grown at the permissive (37 °C) or at the nonpermissive (24 °C) temperature, as described in Experimental Procedures. The proteins in the crude extracts were resolved on a SDS denaturing PAGE gel, and PspA was revealed by immunoblotting analysis.

after a 10 min chase in the MYC-cs strain at the nonpermissive temperature (25 °C). Similarly, PC828ΔH2 cannot efficiently translocate across the membrane even after a 5 min chase in the MYC-cs strain at 25 °C (Figure 3B). Taken together, the above results indicate that the Sec-dependent function of YidC in the MYC-cs mutant is impaired, whereas the Sec-independent function is only marginally affected.

Recently, it has been reported that the expression of the bacterial stress protein PspA is induced after YidC depletion (*5*). Using immunoblot analysis, we confirmed that the level of PspA is elevated in the cs YidC mutant at 24 °C, the nonpermissive temperature (Figure 3C). The level of PspA is markedly higher even at the permissive temperature in the YidC cs mutant compared to the wild-type strain MC1060, which matches the observation that the −5- and −3MPClep mutants are inhibited in membrane insertion at 37 °C.

Increasing the Number of Negative Charges in the Periplasmic Domain of Procoat Makes the Procoat Sec-Dependent. The studies of −4PClep, −5PClep, and −3M-PClep in the MYC-cs and JP325 strains indicate that the periplasmic domain of PClep can determine whether the protein needs the help of the Sec machinery for translocation, in addition to YidC. We are interested in what features of

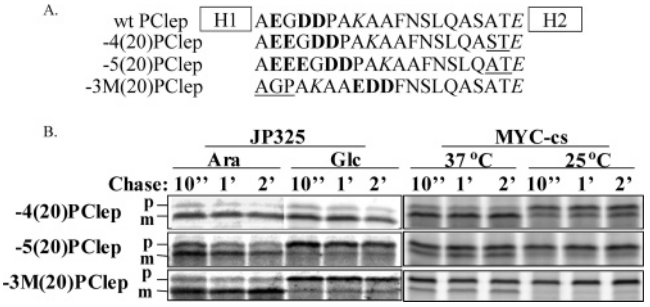

FIGURE 4: Increasing the number of negative charges in the PClep periplasmic domain makes PClep Sec-dependent. (A) The amino acid sequences of the periplasmic domain in wt PClep, −4(20)-PClep, −5(20)PClep, and −3M(20)PClep are depicted. The lengths of periplasmic domains of −4PClep, −5PClep, and −3MPClep were reduced to 20 amino acid residues by removing one, two, and three residues, respectively. The locations where the residues were removed starting from the parent constructs are underlined in the generated constructs, and highlighted in bold are the negatively charged residues. (B) JP325 cells or MYC-cs cells bearing −4(20)PClep, −5(20)PClep, and −3(20)MPClep were grown, labeled, chased, and analyzed as described in the legend of Figure 1. p and m denote the precursor and mature form of PClep, respectively. Ara and Glc indicate growth under arabinose and glucose conditions, respectively.

the periplasmic domain of Procoat can make the protein Sec-dependent. We first fixed the length of the periplasmic domain, and investigated if increasing the number of the negative charges makes Procoat require the Sec complex for insertion. The lengths of the periplasmic domains of −4PClep, −5PClep, and −3MPClep were reduced to 20 amino acid residues, the size of the periplasmic domain of wt PClep (Figure 4A). This was achieved by deletion of one, two, and three residues from the −4-, −5-, and −3MPClep mutants, respectively. We named the new mutants −4(20)PClep, −5(20)PClep, and −3M(20)PClep, respectively. JP325 cells synthesizing these PClep mutants were pulse-chased and analyzed as described in the Figure 2B experiments. We found the radiolabeled −4(20)PClep with one additional negative charge is rapidly processed when SecDF is depleted, whereas the −5(20)Clep with two additional negatively charged residues is not converted to the mature form during the chase (Figure 4B, left panel). Since both proteins have a periplasmic domain of 20 residues in size, the number of negatively charged residues has a profound effect on membrane insertion. The Procoat periplasmic domain with three (wt PClep) or four [−4(20)PClep] negatively charged residues can be translocated very efficiently in a Sec-independent manner, but the five-negative charge mutant [−5(20)PClep] absolutely required the Sec machinery for translocation. Interestingly, the insertion of −3M(20)PClep mutant is also dependent on SecDF (Figure 4B, left panel). In this mutant, there is no additional negatively charged residue in the periplasmic domain (as compared to the wt PClep) and the length of the periplasmic loop is identical to that of the wt PClep, but the three negatively charged residues are moved to the center of the periplasmic domain in the −3M(20)PClep mutant (Figure 4A). This suggests that besides the number of negative charges, the location of the negative charges in the periplasmic loop can also determine whether Procoat needs the assistance of the Sec machinery to promote translocation.

We also tested the insertion of −4(20)PClep, −5(20)-PClep, and −3M(20)PClep in the YidC cs strain, MYC-cs

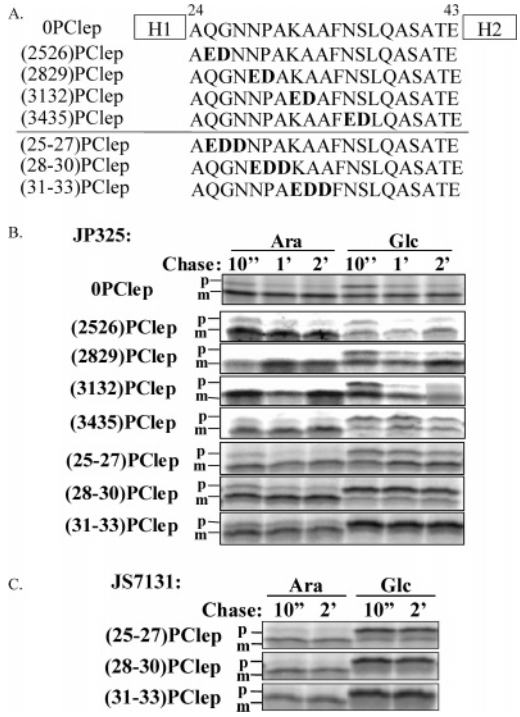

FIGURE 5: Location of the negatively charged residues in the PClep periplasmic domain which determines PClep Sec dependency. (A) The amino acid sequences of the periplasmic domains of the PClep mutants with different locations of the negatively charged residues are indicated. The positions of the first (A24) and last (E43) amino acid residues in the periplasmic domain of 0PClep are indicated. The negatively charged residues that are moved within the periplasmic domain are highlighted in bold. (B) JP325 cells bearing 0PClep or the PClep mutants with different locations of the negatively charged residues described in part A were grown, labeled, chased, and analyzed as described in the legend of Figure 2B. (C) JS7131 cells harboring PClep mutants (in the three-negative charge scanning series) were grown under arabinose (YidC present) or glucose (YidC depleted) conditions. After being radiolabeled with [^{35}S]methionine for 20 s, the cells were chased for 10 s or 2 min with cold methionine. The cells were then TCA precipitated and analyzed by SDS−PAGE and phosphorimaging. In panels B and C, p and m denote the precursor form and mature form of PClep, respectively. Ara and Glc indicate growth under arabinose and glucose conditions, respectively.

(Figure 4B, right panel). Their insertion behavior is similar to that of −4PClep, −5PClep, and −3MPClep in MYC-cs (Figure 2B). The data also reinforce the fact that the Sec-related function of YidC in the cs mutant is impaired, and the Sec-dependent Procoat mutants require SecDF for insertion (Figure 4B).

The Location of the Negatively Charged Residues in the Procoat Periplasmic Domain Also Determines Procoat Sec Dependency. We systematically investigated if the location of the negative charges in the periplasmic loop can determine whether PClep needs the Sec machinery for translocation of the periplasmic loop. We performed positional scanning mutagenesis in the periplasmic loop of PClep by introducing one, two, or three negatively charged residues at different locations in the periplasmic loop of 0PClep, a PClep mutant which has a zero net charge in the periplasmic loop (Figure 5A). We first show that 0PClep is Sec-independent using the JP325 strain; i.e., 0PCLep can still efficiently insert into the membrane when SecDF is depleted (Figure 5B). For the one negative charge scanning, we substituted residue 27, 30, 33, 36, or 39 with an aspartic acid in PClep and tested the

insertion of the PC mutants in JP325. We found that all the mutants can insert into the membrane when SecDF is depleted (data not shown), indicating that they are all Sec-independent. In the two-negative charge series, residues 25 and 26, 28 and 29, 31 and 32, or 34 and 35 were replaced with one glutamic acid and one aspartic acid, respectively (Figure 5A). (2526)PClep, (2829)PClep, and (3132)PClep can efficiently insert into the membrane when SecDF is depleted, and are therefore Sec-independent proteins. In contrast, the membrane insertion of (3435)PClep was more affected by SecDF. This indicates that when two negative charges in the periplasmic loop are moved toward hydrophobic domain 2 (H2) in the mature region of Procoat, the mutants become more Sec-dependent. In the three negatively charged series, the location effect is even more dramatic. When the three negative charges are moved from residues 25−27 to residues 28−30 or 31−33 (Figure 5A), the PClep mutants switch from slightly Sec-dependent to completely Sec-dependent [Figure 5B; see the data for (25−27)PClep, (28−30)PClep, and (31−33)PClep]. When SecDF is depleted (glucose condition, Glc), (25−27)PClep can insert into the membrane efficiently, while (28−30)PClep and (31−33)-PClep are completely blocked (Figure 5B). Taken together, the data indicate that the location of the negatively charged residues in the Procoat periplasmic domain determines Procoat Sec dependency, and the effect of the number and the location of the negative charges in the periplasmic loop for the Sec dependency are additive.

To confirm that the insertion of these PClep mutants is still YidC-dependent, we used the YidC depletion strain, JS7131. Indeed, the insertion of all the PClep mutants described in this report was strictly YidC dependent as tested in the JS7131 strain (data not all shown). Representative data are shown in Figure 5C. The protein constructs (25−27)-PClep, (28−30)PClep, and (31−33)PClep depend on YidC for their membrane insertion (Figure 5C). When YidC is depleted (glucose condition, Glc), the PClep mutants accumulate in their precursor form.

The Length of the Periplasmic Domain Can Contribute to the Procoat Sec Dependency. We next investigated whether the length of the periplasmic loop is also a contributing factor for the Sec dependency of membrane protein insertion of Procoat. We increased the length of the periplasmic loop of PClep to 21, 22, and 23 amino acid residues by adding one, two, and three asparagine residues, respectively, to wt PClep (Figure 6A). Figure 6B shows that PClep with a loop of 21 amino acids (1NPClep) can insert into the membrane, although it is quite dependent on SecDF. When the length of the loop was increased to either 22 and 23 amino acids, the PClep mutants (2NPClep and 3NPClep) display complete Sec dependency; i.e., it cannot insert into the membrane without the Sec machinery. These data indicate that the length of the periplasmic loop can also contribute to the Sec dependency of PClep. As a control, these mutants efficiently inserted into the membrane in wild-type strain MC1060.

A Sec-Dependent Pf3 Coat Is Generated when a Negatively Charged Residue in the Periplasmic Tail of Pf3 Coat Is Moved toward the Hydrophobic Segment. The Procoat mutant data indicate that the characteristic of the periplasmic domain is a key factor that determines whether a Procoat mutant is Sec-dependent. To test how general these features

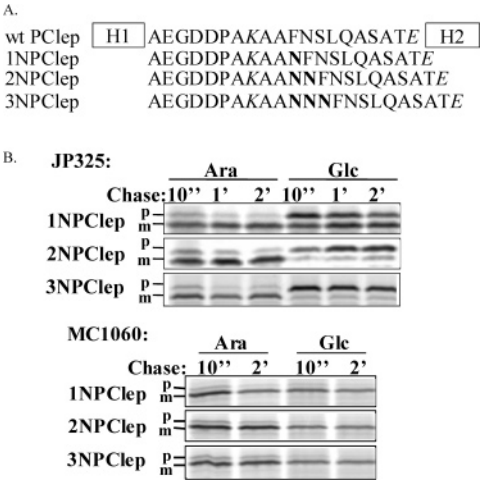

FIGURE 6: Length of the periplasmic domain can dictate the PClep Sec dependency. (A) The amino acid sequences of the periplasmic domains of wt PClep, 1NPClep, 2NPClep, and 3NPClep are shown. 1NPClep, 2NPClep, and 3NPClep have net charges of −3 like wt PClep. The lengths of the periplasmic domains of 1NPClep, 2NPClep, and 3NPClep were increased to 21, 22, and 23 amino acid residues by addition of one, two, and three asparagines (shown in bold), respectively. (B) JP325 or MC1060 cells bearing 1NPClep, 2NPClep, and 3NPClep were grown under arabinose (Ara) and glucose (Glc) conditions, labeled, chased, and analyzed as described in the legend of Figure 2B. p and m denote the precursor form and mature form of PClep, respectively.

of the periplasmic region are, we tested whether another Sec-independent protein, Pf3 coat, can be converted into a Sec-dependent protein. There are two negatively charged residues (Figure 7A, D7 and D18) in the periplasmic N-terminal tail of Pf3 coat (Figure 1). D18 is very close to the hydrophobic domain (H) of Pf3 coat, and its position is similar, with respect to the hydrophobic region, to E43 in the Procoat periplasmic domain (Figure 2A). Like the studies of the Procoat mutants described above, we kept the location of this negatively charged residue (D18) fixed, and moved the other negatively charged residue (D7 in wt Pf3 coat) toward the hydrophobic domain of Pf3 coat. We moved the negative charge from residue 7 (wt Pf3 coat) to residue 11 (Pf3D11 coat) and to residue 16 (Pf3D16 coat), and tested the Sec dependence of insertion of this mutant. Figure 7B shows that when SecDF was depleted, almost all of the wt Pf3 coat was digested by proteinase K (Figure 7B, left panel), which indicates that wt Pf3 coat can efficiently insert without the Sec complex. In contrast, when SecDF was depleted, Pf3D11 coat (Figure 7B, middle panel) and Pf3D16 coat (Figure 7B, right panel) were resistant to the proteinase K digestion even after a 2 min chase with cold methionine, whereas the mature OmpA (a control to monitor the activity of the proteinase K) was completely digested by the protease. ProOmpA and GroEL, a cytosolic protein, were protected from proteinase K digestion (Figure 7B), indicating no lysis of the spheroplasts occurred during the proteolysis. These data indicate that Pf3D11 and Pf3D16 coat become Sec-dependent, as predicted.

We also used the proteinase K protection assay to test the YidC dependency of Pf3D11 and Pf3D16 mutants in strain JS7131. Like the Sec-dependent PClep mutants, these two Sec-dependent Pf3 coat mutants were still strictly YidC-dependent (Figure 7C). When YidC is depleted under glucose conditions, Pf3D11 and Pf3D16 are protected from proteinase

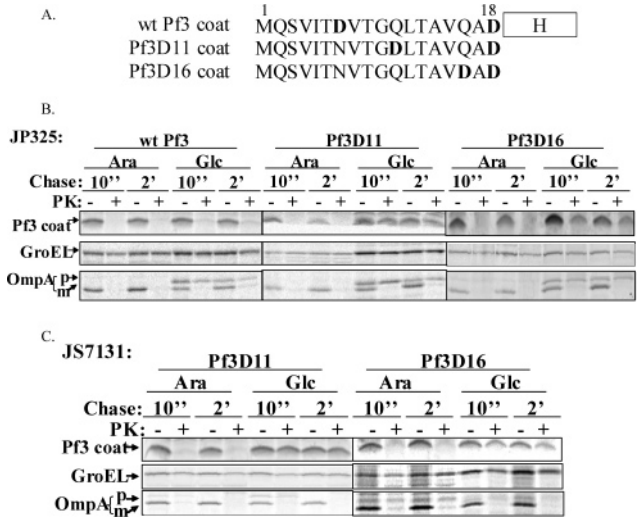

FIGURE 7: Wild-type Pf3 coat is Sec-independent, while Pf3D11 and Pf3D16 are Sec-dependent. (A) The amino acid sequences of the periplasmic domain in wt Pf3 coat, Pf3D11, and Pf3D16 are indicated. The negatively charged residues are highlighted in bold. To move the negatively charged residue at position 7 in the periplasmic tail toward the hydrophobic domain (H), aspartic acid (D) 7 was first mutated to asparagine to remove the charge. Next, glutamine (Q) 11 or 16 was mutated to an aspartic acid, creating Pf3D11 or Pf3D16, respectively. (B) The membrane insertion of wt Pf3 coat, Pf3D11, and Pf3D16 was tested in the SecDF depletion strain, JP325. JP325 cells bearing wt Pf3 coat, Pf3D11, and Pf3D16 were grown in the presence of SecDF (under arabinose condition, Ara) or under SecDF depletion conditions (glucose condition, Glc). (C) The JS7131 strain was used to test the YidC dependency of Pf3D11 and Pf3D16 mutants. JS7131 cells bearing the Pf3 coat mutants were grown under the arabinose (Ara, YidC present) or glucose (Glc, YidC depleted) condition. After growing under the conditions described for panels B and C, the cells were radiolabeled with [^{35}S]methionine for 1 min, and chased for 10 s or 2 min with cold methionine. The cells were then subjected to a proteinase K protection assay to monitor the insertion of Pf3 coat protein. GroEL was used as a cytoplasmic control to show the spheroplasts were intact. OmpA was used as a positive control to measure the efficiency of spheroplast formation; OmpA is not digested in intact cells. p and m of OmpA denote its precursor form and mature form, respectively. Pf3 coat was analyzed by tricine SDS−PAGE and was detected without immunoprecipitation.

K digestion, indicating that they cannot insert into the membrane even after a 2 min chase (Figure 7C, Glc). The data shown above indicate that Pf3D11 and Pf3D16 are both strictly Sec-dependent and YidC-dependent.

DISCUSSION

In this work, we show that (1) SecDF, which spans YidC to SecYEG, is essential for the insertion of the Sec-dependent M13 Procoat and Pf3 coat variants, (2) the Sec-related function of YidC is strongly inhibited in the cold-sensitive YidC mutant, and (3) subtle changes in the periplasmic regions of YidC-dependent proteins can make the protein strictly Sec-dependent. Taken together, a model describing how YidC accomplishes its insertion role in concert with the Sec machinery is proposed below.

Previously, SecDF has been shown to facilitate the membrane translocation of exported proteins *in vivo*. Our results with the mutant viral coat proteins now suggest that SecDF plays an important role in the insertion of some membrane proteins. In the SecDF depletion strain, these proteins were not translocated and accumulated in the cytoplasm. This suggests that SecDF contributes to the translocation step possibly in conjunction with YidC and SecYEG.

In contrast to wild-type PClep, all Sec-dependent PClep mutants studied in this work were strongly inhibited for membrane insertion in the cold-sensitive YidC mutant MYC-cs, suggesting that MYC-cs is strongly impaired in its Sec-related function. The colony formation of the MYC-cs strain is inhibited at 25 °C but normal at 37 °C (*32*). Notably, insertion of the Sec-dependent PClep proteins was even inhibited at the permissive temperature. The YidC cs mutant is not completely functional at the permissive temperature as the level of the stress protein PsbA is increased at 37 °C compared to that of the wild-type strain (Figure 3C). Despite this, the cs YidC mutant still has sufficient activity to translocate the wild-type YidC protein at the permissive temperature. However, the cs mutation also impairs YidC in its function to insert certain Sec-independent proteins. Like −5PClep and −3MPClep, −4PClep is also blocked in the MYC-cs strain at the nonpermissive temperature. However, unlike −5PClep and −3MPClep, which are Sec-dependent, −4PClep is Sec-independent (Figure 2). We conclude that the activity of the cs-YidC is marginally affected for its Sec-independent function. The mutations in the cs YidC mutant are within periplasmic loop P1, replacing residues 24−27 with an IEGR sequence and introducing the LEVLFQGP sequence between L333 and L339 (*32*).

Starting from −4PClep, −5PClep, and −3MPClep, we systematically studied what features in the Procoat periplasmic domain make the Procoat mutants require the YidC Sec-related function and become Sec-dependent. We discovered three features (number and location of the negatively charged residues, and the size) in the PClep periplasmic loop that contributes to the Sec dependency. These features may not constitute all the factors that contribute to Sec dependency, and other features that can convert Procoat into a Sec-dependent protein may exist. We applied one of the features, i.e., moving negative charges in the periplasmic domain toward the hydrophobic domain in the Pf3 coat protein, and successfully converted the Sec-independent Pf3 coat into a Sec-dependent protein. It is the first time a Sec-dependent Pf3 coat mutant was found. This suggests that the rules that determine Sec dependency from our studies with PClep can be extended to other proteins.

Our hypothesis is that the function of the Sec complex for the Sec-dependent Procoat mutants is to facilitate the translocation of the periplasmic domain since all of the mutations described in this paper which make Procoat Sec-dependent occur in the periplasmic domain of Procoat. The translocation ability of YidC is quite limited. YidC alone can only mediate the translocation of periplasmic domains of the best substrates, such as wt Procoat. The translocation of the wt Procoat periplasmic domain may be a result of the YidC-mediated formation of the transmembrane configuration of the Procoat hydrophobic domains, thereby promoting translocation of the central hydrophilic region across the membrane. This effect may not be strong enough to translocate unfavorable periplasmic domains. When the character of the periplasmic domain (such as the location or the number of negatively charged residues) changes, the energy barrier to translocation of the periplasmic domain may become too high. In this case, YidC itself cannot translocate

the periplasmic domains of the Procoat mutants, and it needs the Sec machinery to facilitate translocation. SecDF of the Sec machinery helps YidC to promote the translocation of the unfavorable periplasmic domain of the Procoat mutants. This suggests that the Sec-dependent Procoat variants insert at the junction between YidC and SecYEG with SecDF assisting in the translocation process.

The membrane insertion of Sec-dependent −5PClep and −3MPClep requires SecDF and SecE, as well as YidC for insertion. Their strict YidC dependence differs from that of other Sec-dependent proteins such as FtsQ that do not absolutely require YidC for insertion (*15*). These PClep mutants are also different from the other YidC-dependent proteins such as Procoat which do not require Sec machinery (*2*).

On the basis of the data presented in this report, we propose there are three different functions or requirements of YidC in protein translocation. YidC functions as an accessory component for certain Sec-dependent proteins, whereas the Sec complex plays a central role in membrane insertion and translocation of the hydrophilic domain. YidC plays only a minor role. YidC has this function for the insertion of the FtsQ and leader peptidase (Lep). For another group of Sec substrates, YidC plays a central role in membrane insertion and the limited translocation capacity of YidC itself cannot translocate the periplasmic domains of the membrane protein across the membrane. The Sec machinery, including the SecDF, is required for the translocation process as well. Examples of membrane proteins that use this pathway are −5PClep, −3MPClep, Pf3D11, and Pf3D16. The periplasmic domains of these membrane proteins make these proteins less than optimal as substrates of YidC. Translocation of these proteins by YidC alone is energetically unfavorable. The third function of YidC is relevant for Sec-independent proteins. YidC mediates the insertion of the hydrophobic domains of a YidC-dependent membrane such as wild-type Procoat. However, different than the substrates that use YidC in the second function, the periplasmic domains of these substrates are translocated by YidC itself. The features of the periplasmic domains of these proteins may be the most favorable for YidC-mediated translocation.

ACKNOWLEDGMENT

We thank Daniel Ritz and Jonathan Beckwith for providing the JP325 strain, Chris Murphy for the CM124 strain, and Jan Tommassen for antisera against PspA. We also thank Huai-Chun Chen for the help in some experiments.

REFERENCES

1. Samuelson, J. C., Chen, M., Jiang, F., Moller, I., Wiedmann, M., Kuhn, A., Phillips, G. J., and Dalbey, R. E. (2000) YidC mediates membrane protein insertion in bacteria, *Nature 406*, 637−641.
2. Samuelson, J. C., Jiang, F., Yi, L., Chen, M., de Gier, J. W., Kuhn, A., and Dalbey, R. E. (2001) Function of YidC for the insertion of M13 procoat protein in *E. coli*: Translocation of mutants that show differences in their membrane potential dependence and Sec requirement, *J. Biol. Chem. 276*, 34847−34852.
3. Chen, M., Xie, K., Jiang, F., Yi, L., and Dalbey, R. E. (2002) YidC, a newly defined evolutionarily conserved protein, mediates membrane protein assembly in bacteria, *Biol. Chem. 383*, 1565−1572.
4. Chen, M., Samuelson, J. C., Jiang, F., Muller, M., Kuhn, A., and Dalbey, R. E. (2002) Direct interaction of YidC with the Sec-independent Pf3 coat protein during its membrane protein insertion, *J. Biol. Chem. 277*, 7670−7675.
5. van Der Laan, M., Urbanus, M. L., Ten Hagen-Jongman, C. M., Nouwen, N., Oudega, B., Harms, N., Driessen, A. J., and Luirink, J. (2003) A conserved function of YidC in the biogenesis of respiratory chain complexes, *Proc. Natl. Acad. Sci. U.S.A. 100*, 5801−5806.
6. Yi, L., Celebi, N., Chen, M., and Dalbey, R. E. (2004) Sec/SRP requirements and energetics of membrane insertion of subunits a, b, and c of the *Escherichia coli* F1F0 ATP synthase, *J. Biol. Chem. 279*, 39260−39267.
7. Yi, L., Jiang, F., Chen, M., Cain, B., Bolhuis, A., and Dalbey, R. E. (2003) YidC is strictly required for membrane insertion of subunits a and c of the F_1F_0ATP synthase and SecE of the SecYEG translocase, *Biochemistry 42*, 10537−10544.
8. Froderberg, L., Houben, E., Samuelson, J. C., Chen, M., Park, S. K., Phillips, G. J., Dalbey, R., Luirink, J., and De Gier, J. W. (2003) Versatility of inner membrane protein biogenesis in *Escherichia coli*, *Mol. Microbiol. 47*, 1015−1027.
9. Serek, J., Bauer-Manz, G., Struhalla, G., Van Den Berg, L., Kiefer, D., Dalbey, R., and Kuhn, A. (2004) *Escherichia coli* YidC is a membrane insertase for Sec-independent proteins, *EMBO J. 23*, 294−301.
10. Van Der Laan, M., Bechtluft, P., Kol, S., Nouwen, N., and Driessen, A. J. (2004) F1F0 ATP synthase subunit c is a substrate of the novel YidC pathway for membrane protein biogenesis, *J. Cell Biol. 165*, 213−222.
11. Stuart, R. A., and Neupert, W. (2000) Making membranes in bacteria [news], *Nature 406*, 575, 577.
12. Stuart, R. (2002) Insertion of proteins into the inner membrane of mitochondria: The role of the Oxa1 complex, *Biochim. Biophys. Acta 1592*, 79−87.
13. Luirink, J., Samuelsson, T., and de Gier, J. W. (2001) YidC/Oxa1p/Alb3: Evolutionarily conserved mediators of membrane protein assembly, *FEBS Lett. 501*, 1−5.
14. Kuhn, A., Stuart, R., Henry, R., and Dalbey, R. E. (2003) The Alb3/Oxa1/YidC protein family: Membrane-localized chaperones facilitating membrane protein insertion? *Trends Cell Biol. 13*, 510−516.
15. Urbanus, M. L., Scotti, P. A., Froderberg, L., Saaf, A., de Gier, J. W., Brunner, J., Samuelson, J. C., Dalbey, R. E., Oudega, B., and Luirink, J. (2001) Sec-dependent membrane protein insertion: Sequential interaction of nascent FtsQ with SecY and YidC, *EMBO Rep. 2*, 524−529.
16. van der Laan, M., Nouwen, N., and Driessen, A. J. (2004) SecYEG proteoliposomes catalyze the $\Delta\psi$-dependent membrane insertion of FtsQ, *J. Biol. Chem. 279*, 1659−1664.
17. Wolfe, P. B., Rice, M., and Wickner, W. (1985) Effects of two sec genes on protein assembly into the plasma membrane of *Escherichia coli*, *J. Biol. Chem. 260*, 1836−1841.
18. Houben, E. N., Scotti, P. A., Valent, Q. A., Brunner, J., de Gier, J. L., Oudega, B., and Luirink, J. (2000) Nascent Lep inserts into the *Escherichia coli* inner membrane in the vicinity of YidC, SecY and SecA, *FEBS Lett. 476*, 229−233.
19. Koch, H. G., Hengelage, T., Neumann-Haefelin, C., MacFarlane, J., Hoffschulte, H. K., Schimz, K. L., Mechler, B., and Muller, M. (1999) *In vitro* studies with purified components reveal signal recognition particle (SRP) and SecA/SecB as constituents of two independent protein-targeting pathways of *Escherichia coli*, *Mol. Biol. Cell 10*, 2163−2173.
20. Koch, H. G., Moser, M., Schimz, K. L., and Muller, M. (2002) The integration of YidC into the cytoplasmic membrane of *Escherichia coli* requires the signal recognition particle, SecA and SecYEG, *J. Biol. Chem. 277*, 5715−5718.
21. Urbanus, M. L., Froderberg, L., Drew, D., Bjork, P., de Gier, J. W., Brunner, J., Oudega, B., and Luirink, J. (2002) Targeting, insertion, and localization of *Escherichia coli* YidC, *J. Biol. Chem. 277*, 12718−12723.
22. Traxler, B., and Murphy, C. (1996) Insertion of the polytopic membrane protein MalF is dependent on the bacterial secretion machinery, *J. Biol. Chem. 271*, 12394−12400.
23. Newitt, J. A., and Bernstein, H. D. (1998) A mutation in the *Escherichia coli* secY gene that produces distinct effects on inner membrane protein insertion and protein export, *J. Biol. Chem. 273*, 12451−12456.

24. Ito, K., and Akiyama, Y. (1991) *In vivo* analysis of integration of membrane proteins in *Escherichia coli*, *Mol. Microbiol. 5*, 2243−2253.
25. Akimaru, J., Matsuyama, S., Tokuda, H., and Mizushima, S. (1991) Reconstitution of a protein translocation system containing purified SecY, SecE, and SecA from *Escherichia coli*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 6545−6549.
26. Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J., and Wickner, W. (1990) The purified *E. coli* integral membrane protein SecY/E is sufficient for reconstitution of SecA-dependent precursor protein translocation, *Cell 62*, 649−657.
27. Van den Berg, B., Clemons, W. M., Jr., Collinson, I., Modis, Y., Hartmann, E., Harrison, S. C., and Rapoport, T. A. (2004) X-ray structure of a protein-conducting channel, *Nature 427*, 36−44.
28. Pogliano, J. A., and Beckwith, J. (1994) SecD and SecF facilitate protein export in *Escherichia coli*, *EMBO J. 13*, 554−561.
29. Duong, F., and Wickner, W. (1997) The SecDFyajC domain of preprotein translocase controls preprotein movement by regulating SecA membrane cycling, *EMBO J. 16*, 4871−4879.
30. Scotti, P. A., Urbanus, M. L., Brunner, J., de Gier, J. W., von Heijne, G., van der Does, C., Driessen, A. J., Oudega, B., and Luirink, J. (2000) YidC, the *Escherichia coli* homologue of mitochondrial Oxa1p, is a component of the Sec translocase, *EMBO J. 19*, 542−549.
31. Nouwen, N., and Driessen, A. J. (2002) SecDFyajC forms a heterotetrameric complex with YidC, *Mol. Microbiol. 44*, 1397−1405.
32. Chen, M., Xie, K., Nouwen, N., Driessen, A. J., and Dalbey, R. E. (2003) Conditional Lethal Mutations Separate the M13 Procoat and Pf3 Coat Functions of YidC: Different YidC structural requirements for membrane protein insertion, *J. Biol. Chem. 278*, 23295−23300.
33. Nagamori, S., Smirnova, I. N., and Kaback, H. R. (2004) Role of YidC in folding of polytopic membrane proteins, *J. Cell Biol. 165*, 53−62.
34. Cao, G., Kuhn, A., and Dalbey, R. E. (1995) The translocation of negatively charged residues across the membrane is driven by the electrochemical potential: Evidence for an electrophoresis-like membrane transfer mechanism, *EMBO J. 14*, 866−875.
35. Economou, A., Pogliano, J. A., Beckwith, J., Oliver, D. B., and Wickner, W. (1995) SecA membrane cycling at SecYEG is driven by distinct ATP binding and hydrolysis events and is regulated by SecD and SecF, *Cell 83*, 1171−1181.
36. Kuhn, A. (1988) Alterations in the extracellular domain of M13 procoat protein make its membrane insertion dependent on *secA* and *secY*, *Eur. J. Biochem. 177*, 267−271.
37. Kuhn, A., Zhu, H. Y., and Dalbey, R. E. (1990) Efficient translocation of positively charged residues of M13 procoat protein across the membrane excludes electrophoresis as the primary force for membrane insertion, *EMBO J. 9*, 2385−2388, 2429, 3413 (published erratum).
38. Schuenemann, T. A., Delgado-Nixon, V. M., and Dalbey, R. E. (1999) Direct evidence that the proton motive force inhibits membrane translocation of positively charged residues within membrane proteins, *J. Biol. Chem. 274*, 6855−6864.
39. Hashemzadeh-Bonehi, L., Mehraein-Ghomi, F., Mitsopoulos, C., Jacob, J. P., Hennessey, E. S., and Broome-Smith, J. K. (1998) Importance of using lac rather than ara promoter vectors for modulating the levels of toxic gene products in *Escherichia coli*, *Mol. Microbiol. 30*, 676−678.
40. Gerard, F., Pradel, N., Ye, C., Ize, B., Yi, L., Xu, J., Dalbey, R. E., and Wu, L. F. (2004) Putative membrane assembly of EtpM-colicin V chimeras, *Biochimie 86*, 283−286.
41. Miller, J. H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Plainview, NY.
42. de Gier, J. W., Scotti, P. A., Saaf, A., Valent, Q. A., Kuhn, A., Luirink, J., and von Heijne, G. (1998) Differential use of the signal recognition particle translocase targeting pathway for inner membrane protein assembly in *Escherichia coli* [see comments], *Proc. Natl. Acad. Sci. U.S.A. 95*, 14646−14651.

BI047418K