Defining the Regions of *Escherichia coli* YidC That Contribute to Activity*

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The YidC/Oxa1/Alb3 family of proteins catalyzes membrane protein insertion in bacteria, mitochondria, and chloroplasts. In this study, we investigated which regions of the bacterial YidC protein are important for its function in membrane protein biogenesis. In *Escherichia coli*, YidC spans the membrane six times, with a large 319-residue periplasmic domain following the first transmembrane domain. We found that this large periplasmic domain is not required for YidC function and that the residues in the exposed hydrophilic loops or C-terminal tail are not critical for YidC activity. Rather, the five C-terminal transmembrane segments that contain the three consensus sequences in the YidC/Oxa1/Alb3 family are important for its function. However, by systematically replacing all the residues in transmembrane segment (TM) 2, TM3, and TM6 with serine and by swapping TM4 and TM5 with unrelated transmembrane segments, we show that the precise sequence of these transmembrane regions is not essential for in vivo YidC activity. Single serine mutations in TM2, TM3, and TM6 impaired the membrane insertion of the Sec-independent procoat-leader peptidase protein. We propose that the five C-terminal transmembrane segments of YidC function as a platform for the translocating substrate protein to support its insertion into the membrane.

In prokaryotes and eukaryotes, the YidC/Oxa1/Alb3 family of proteins mediates membrane protein insertion. In bacteria, YidC mediates the membrane insertion of a number of proteins (for review, see Refs. 1 and 2). YidC has been found associated with the SecYEGDF-YajC complex and to interact with Sec-dependent membrane proteins during membrane insertion (3–5). However, *Escherichia coli* depleted of YidC is mildly defective in the insertion of Sec-dependent membrane proteins (6, 7), but is blocked in the insertion of proteins that do not use the Sec translocase, such as the M13 phage procoat protein and the Ph3 phage coat protein (6, 8, 9). These Sec-independent proteins were previously thought not to require a proteinaceous factor (10–12). Similarly, in mitochondria, Oxa1 is required for the insertion of a subset of proteins that are inserted into the mitochondrial inner membrane from the matrix compartment (13–15). These inner membrane proteins include both mitochondrial encoded proteins that are synthesized in the matrix and nuclear encoded proteins that are first imported into mitochondria from the cytoplasm (for review, see Ref. 16). In chloroplasts, Alb3, the Oxa1 homolog, is needed for the insertion of certain light-harvesting chlorophyll-binding proteins into the thylakoid membrane (17, 18).

The membrane topology of the *E. coli* YidC protein is different from that of Oxa1 and Alb3. Unlike Oxa1 and Alb3, which span the membrane five times with an N-terminal luminal tail of 100 residues or less (19), YidC spans the membrane six times and, following the first transmembrane region, has a large N-terminal domain of 319 residues in the periplasm (see Fig. 1) (20). The second, third, fifth, and sixth membrane-spanning regions of YidC contain conserved residues in the YidC/Oxa1/Alb3 family (21). In addition, cytoplasmic loop C1 and periplasmic loop P2 of *E. coli* YidC (see Fig. 1) contain conserved hydrophilic regions that have been proposed to be important for substrate recognition (21).

As a first step in defining the functional domains of *E. coli* YidC, we have mapped the regions of the protein that are important for YidC activity. We found that the large N-terminal periplasmic domain, periplasmic loops, and cytoplasmic regions of YidC are not directly involved in function, but that the five C-terminal transmembrane regions play an important role. This important role of the five transmembrane regions is consistent with sequence alignment analysis of the Oxa1 family members, which almost all have five transmembrane segments (21). In particular, we identified six YidC single serine substitution mutants in the transmembrane regions that had impaired activity in the membrane insertion of procoat-leader peptidase protein. We propose that the C-terminal transmembrane segments of YidC function as a platform or framework in membrane protein insertion.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—*E. coli* strain JS7131 (MC1060, ΔyidC, attB::R6Kori, ParaBADyidC (SpeC)) was constructed in our laboratory as described previously (6). Plasmid pMS119, containing the tac promoter and lacIq, was used to overproduce the M13 procoat protein with a leader peptidase domain (the last 103 residues of leader peptidase) attached to the C terminus (PC-Lep). The *yidC* open reading frame, along with the 370-bp upstream region including the promoter and ribosome-binding site, was cloned into pACYC184, a low copy number plasmid from New England Biolabs Inc. The resulting pACYC184-yidC plasmid was then used to make all YidC mutants in this study.

The abbreviations used are: PC-Lep, M13 procoat protein with a leader peptidase domain (the last 103 residues of leader peptidase) attached to the C terminus; TM, transmembrane segment.
work, yidC contains a natural PstI (CTGCAG) site starting at the position corresponding to amino acid 147, and we introduced a second PstI site at codon 25 such that CTG encodes amino acid 25. Then, the PstI site at amino acid 147 was removed by a silent mutation, and another PstI site was added, with CTG codons introduced at amino acids 83, 214, 278, 323, and 332, respectively. The plasmids with the two PstI sites were digested with the PstI enzyme and then religated, yielding the yidC deletion mutants Δ25–83, Δ25–147, Δ25–214, Δ25–278, Δ25–323, and Δ25–332. For the other deletion mutants, oligonucleotide primers were designed complementary to the flanking regions of the deletion. To replace TM4 and TM5 of YidC with leader peptidases H1 and H2, a DNA fragment containing H1 or H2 was inserted into the yidC gene after the sequence coding for TM4 or TM5. Site-directed mutagenesis (Stratagene) was carried out to remove the extra part of leader peptidase. For all other yidC mutants, site-directed mutagenesis was used to replace the wild-type codons with the desired amino acid codons.

**Test of Complementation**—Competent JS7131 cells were incubated on ice with the plasmids containing different yidC mutants for 15 min and heat-shocked at 37 °C for 3 min. Fresh LB medium was added and incubated at 37 °C for 30 min. The same amount of the culture was spread on LB plates with 0.2% arabinose or 0.2% glucose and incubated at 37 °C for 16–18 h. Cells that grew on both arabinose and glucose plates were recorded as “complementation-positive,” and cells that grew only on arabinose plates were recorded as “complementation-negative.”

**Signal Peptide Processing Assay**—Strain JS7131 transformed with both pACYC184-yidCmutant and pMS119-PC-Lep was grown overnight in LB medium with 0.2% arabinose. After washing twice with fresh LB medium, the overnight cultures were back-diluted 1:50 into 1 ml of LB medium containing 0.2% arabinose and 1% glucose and grown for another 0.5 h. At this point, isopropyl-β-D-thiogalactopyranoside (final concentration of 1 mM) was added to the culture to induce the expression of PC-Lep. After 5 min, the cells were pelleted and added to 2 ml of 25% trichloroacetic acid. The samples were subjected to SDS-PAGE using a 15% polyacrylamide gel. YidC mutants were pelleted and added to 2 ml of 25% trichloroacetic acid. The samples were subjected to SDS-PAGE using a 15% polyacrylamide gel. YidC mutants could be measured when strain JS7131 was transformed with plasmids expressing different YidC mutants under conditions in which the arabinose-inducible chromosomal YidC was not expressed.

**Detection of YidC Mutants by Immunoblot Assay**—Strain JS7131 with the YidC mutants in the pACYC184 vector was grown overnight in LB medium containing 0.2% arabinose. After washing the cells with fresh LB medium twice, the overnight cultures were back-diluted 1:50 into 1 ml of LB medium with 0.2% arabinose and 1% glucose and grown to deplete wild-type YidC. After 2.5 h of growth, JS7131 cells producing the YidC mutants were pelleted and added to 2× SDS-PAGE loading dye. Samples were analyzed by SDS-PAGE using a 15% polyacrylamide gel. YidC proteins were detected by immunoblotting using antisera that was prepared against the C terminus of YidC and the ECL Western blot detection kit (Amersham Biosciences).

**RESULTS**

**In Vivo Assay of YidC Activity**—To examine the activity of the YidC mutants, we used two approaches. Both approaches took advantage of the arabinose-dependent JS7131 strain, which has the yidC gene under the control of the araBAD promoter (6). Growth of the JS7131 strain is strictly arabinose-dependent, i.e. growth on LB medium supplemented with arabinose is normal, but not on LB medium with glucose. In the first approach, we cloned the YidC mutants into pACYC184 under the control of the endogenous promoter of yidC and investigated whether the mutant YidC protein could complement the growth defect of the YidC depletion strain when the cells were grown on glucose. In the second approach, we transformed the JS7131 strain with pMS119 encoding the PC-Lep gene and with the pACYC184 plasmid containing each yidC mutant. There are two reasons for choosing PC-Lep as the model protein to test the membrane insertion function of the YidC mutants. One is that PC-Lep insertion is strictly dependent on YidC (8). The other reason is that the PC-Lep insertion can be easily detected using the leader peptidase processing assay, allowing us to test a large number of YidC mutants. It should be noted that we verified the identities of the precursor and mature forms of PC-Lep bands by +/− induction and by immunoprecipitation (data not shown). The PC-Lep bands were easily visualized because the protein is highly expressed in the pMS119 vector and because there were no other highly expressed proteins at the same position on the SDS-polyacrylamide gel. Using the two-plasmid system, we could measure the in vivo activity of the YidC mutant by examining PC-Lep processing after growing the cells in the presence of glucose for several generations, which results in repression of wild-type YidC synthesis. When the plasmid-encoded YidC mutant was inactive (see Fig. 3B, for example), PC-Lep was inhibited in membrane insertion. In contrast, when strain JS7131 had a functional yidC gene on the plasmid, we found that PC-Lep was processed in cells growing on glucose. Therefore, the activity of YidC mutants could be measured when strain JS7131 was transformed with plasmids expressing different YidC mutants under conditions in which the arabinose-inducible chromosomal YidC was not expressed.

**YidC Is Still Functional When Most of the Large N-terminal Periplasmic Domain Is Deleted**—Because the YidC homologs in mitochondria and chloroplasts as well as in many bacteria have much shorter luminal domains (1, 21), we investigated whether the large periplasmic domain of YidC (loop P1) (Fig. 1) is functional in E. coli YidC. To examine the activity of YidC mutants, site-directed mutagenesis (Stratagene) was carried out to remove the extra part of leader peptidase. For all other yidC mutants, site-directed mutagenesis was used to replace the wild-type codons with the desired amino acid codons.

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**YidC Is Still Functional When Most of the Large N-terminal Periplasmic Domain Is Deleted**—Because the YidC homologs in mitochondria and chloroplasts as well as in many bacteria have much shorter luminal domains (1, 21), we investigated whether the large periplasmic domain of YidC (loop P1) (Fig. 1) is required for activity. To address this question, we made a series of periplasmic domain deletion mutants: Δ25–83, Δ25–147, Δ25–214, Δ25–278, Δ25–323, and Δ25–332 (the numbers in the mutant names indicate the positions of amino acid residues in YidC) (Fig. 2A). Immunoblot analysis using anti-YidC antisera prepared against a C-terminal peptide was performed to examine the size and expression of the YidC deletion mutants (Fig. 2B). As expected, as the deletions became bigger, the expressed YidC mutant proteins decreased in size. Proteolytic fragments of YidC were evident for the Δ25–83 and Δ25–147 mutants. However, no obvious expressed protein was evident for the Δ25–323 mutant; this YidC mutant may overlap with the background band, or it is poorly expressed. Fig. 3 shows that most of periplasmic loop P1 could be deleted without a detrimental effect on YidC function. Except for the largest deletion (Δ25–332), all the loop P1 deletion mutants complemented the YidC deletion strain (Fig. 3A) and stimulated the insertion of PC-Lep when wild-type yidC controlled by araBAD was not expressed (Fig. 3B). Even the Δ25–332 mutant was functional in E. coli, showing that 92% of the periplasmic domain is dispensable for function. The results of this deletion
the cells were shifted to M9 minimal medium and grown for glucose and grown to deplete wild-type YidC. After 3 h of growth in LB, the overnight culture was back-diluted 1:50 in LB medium with 0.2% arabinose. After washing the cells twice with fresh LB medium, the overnight cultures were back-diluted 1:50 into 1 ml of LB medium with 0.2% glucose and grown for 2.5 h. The cells were then pelleted and resuspended in 2× loading buffer. The samples were analyzed by SDS-PAGE and immunoblotting using antiserum against a C-terminal peptide of YidC. YidCWT, wild-type YidC.

Alanine scanning mutagenesis analysis of the periplasmic region (amino acids 324–342). A, shown is a summary of the alanine mutants in periplasmic loop P1. B, JS7131, the YidC depletion strain, expressing each of the YidC mutants was grown and pulse-labeled with Tran35S-label as described in the legend of Fig. 3B. Previously, we showed that the amount of YidC in the JS7131 strain grown in arabinose medium is approximately the same as that of wild-type YidC in the parent E. coli strain MC1060 (6).

To determine whether a specific sequence between residues 324 and 342 in periplasmic loop P1 is necessary for YidC activity, we used alanine scanning mutagenesis. Remarkably, all the Ala mutants were active (Fig. 4), showing that there are no essential residues in the remaining 18 residues of the YidC periplasmic domain. The results emphasize that the periplasmic domain is not critical per se for YidC function in E. coli.

The Other Hydrophilic Loops and the C-terminal Tail Do Not Contain Residues That Are Important for Substrate Binding or Catalysis—Recently, it has been proposed that, in the Oxa1 family of proteins, there are conserved hydrophilic regions that may play a role in substrate recognition (21). Two of these conserved hydrophilic regions are located in cytosolic loop C1 between TM2 and TM3, and the third conserved hydrophilic region is located in periplasmic loop P2 between TM3 and TM4 of E. coli YidC (Fig. 1). To test whether the hydrophilic regions contain important information for substrate recognition, we deleted these regions as well as the other hydrophilic domains in the protein. We found that deletion of the entire cytoplasmic loop C1 (residues 371–416; ΔLoopC1) and cytosolic loop C2 (residues 482–493; ΔLoopC2) had no effect on YidC activity. First, both ΔLoopC1 and ΔLoopC2 complemented the YidC depletion strain (Fig. 5A); and second, they stimulated the insertion of PC-Lep (Fig. 5B), as PC-Lep was converted to the mature form. Similarly, deletion of the short cytoplasmic C-terminal tail (residues 535–548; ΔC) had no significant effect on YidC function (Fig. 5). In contrast, deletion of periplasmic loop P2 (residues 447–463; ΔLoopP2) inactivated the protein. No processing of PC-Lep was observed for this mutant. Because the deletion could inactivate the protein due to a structural change, we used a second approach involving alanine scanning mutagenesis. Four alanine mutants were made in periplasmic loop P2 to test whether the residues in this loop are critical for YidC function (Fig. 5). All the Ala mutants were made in periplasmic loop P2 to test whether the residues in this loop are critical for YidC function (Fig. 5).

The results demonstrate that if periplasmic loop P2 plays a role in substrate binding, then this domain does not require a precise sequence for substrate recognition. Finally, deletion analysis and alanine scanning mutagenesis were performed on periplasmic loop P3 (residues 510–512; ΔLoopP3) (Fig. 5). Alanine scanning mutagenesis of periplasmic loop P3 shows that the residues in this loop are not critical for YidC function. Analysis of alanine mutants in periplasmic loop P3 shows that the residues in this loop are not critical for YidC function. Analysis of alanine mutants in periplasmic loop P3 shows that the residues in this loop are not critical for YidC function.
though deletion of the loop impaired processing of PC-Lep and prevented the YidC mutant from complementing the YidC deletion strain, the loop did not contain any residues essential for activity of YidC; this short loop could be functionally replaced by polyalanine (Fig. 5).

The Five C-terminal Transmembrane Segments Are Critical for YidC Activity—Because almost all YidC homologs have five transmembrane segments (21), deletion analysis was performed to test the importance of the five C-terminal transmembrane segments for YidC function. We reported previously that YidC is still functional when TM1 is replaced with a cleavable signal peptide (22). This suggests that TM1 of YidC functions as an uncleaved signal sequence and is important for membrane insertion of YidC, but not for YidC activity. We now investigated whether the other five transmembrane segments are important for YidC activity. Three deletion mutants were initially made (Fig. 6A). First, TM6 was deleted along with the cytoplasmic tail (ΔTM6C). Second, TM5 and TM6 were deleted (ΔTM5+6). Third, TM3 and TM4 (including loop P2) were deleted (ΔTM3+4). All these mutants were inactive; no complementation was observed (Fig. 6A), and they were not capable of stimulating PC-Lep membrane insertion (Fig. 6B). In addition, a small deletion in TM2 or TM3 was made (ΔTM2 has WGFPSIII deleted, and ΔTM3 has VNPLGCF deleted). Also these deletions inactivated YidC, indicating that TM2 and TM3 are important for YidC activity. To confirm that the complementation-negative YidC deletion constructs were expressed, we examined the YidC intracellular levels by immunoblotting. The JS7131 strain was transformed with the YidC constructs and grown in either arabinose medium to express wild-type YidC or control, strain JS7131 with an empty vector was grown under glucose conditions as the deletions are so small (seven residues for ΔTM2 and eight residues for ΔTM3), showing they were indeed expressed (Fig. 7). As expected, ΔTM3+4 and ΔTM5+6, with deletions of 65 and 42 residues, respectively, were detected on the SDS-polyacrylamide gel running at a molecular mass below that of the wild-type protein. This immunoblot study rules out that the deletions did not result in poor expression or instability of the YidC proteins. Nevertheless, the C-terminal transmembrane domains in YidC may be important for maintaining the overall structure of the protein or for correct membrane insertion of the YidC protein. These deletion studies show that, to maintain the function of YidC, the five C-terminal transmembrane segments need to be present. We therefore further investigated whether specific sequences in the five transmembrane domains are necessary for YidC function.

YidC Can Tolerate Most of the Serine Mutations in Conserved Membrane-spanning Regions 2, 3, and 6—The recent analysis by Yen et al. (21) of the Oxa1 protein family of proteins found three consensus sequences that were derived from the most conserved regions of these proteins. Three consensus sequences were found. The first is in conserved TM2, the second in TM3, and the third in TM5 and TM6 of E. coli YidC. Because we found that TM5 can be replaced by an unrelated transmembrane segment (see below), we focused on the consensus sequences in TM2, TM3, and TM6. The consensus sequence residues in TM2, TM3, and TM6 of E. coli YidC are shown in boldface in the legends of Table I, II, and III, respectively.

To test the importance of the conserved residues in TM2, TM3, and TM6 as well as other residues in these membrane-spanning regions, we made a number of site-directed mutants. First, serine scanning mutagenesis was performed. Typically, three or more serine residues were introduced consecutively into the transmembrane segments. Serine was chosen because most of the conserved residues are hydrophobic and reside in membrane-spanning regions. Therefore, the polar serine residue should allow the transmembrane region to insert into the membrane, whereas if the conserved residue or its hydrophobicity is critical for activity, the serine mutation should impair the activity of the mutant. The majority of the serine mutants in TM2 were functional (Table I). They complemented the YidC deletion strain and promoted the insertion of PC-Lep (Table I). Intriguingly, two of the YidC mutants in which serines were substituted for residues 343–345 or 361–365 were not functional (Table I). These serine mutants did not complement the growth defect of the YidC deletion strain and were defective in promoting membrane insertion of PC-Lep (Table I; see Fig. 8A
The sequence of TM2 is I343LLKWHISFVGWGSFMHVTFIVRGSIMY<sup>770</sup>. The boldface residues are part of the first consensus sequence in the Oxal protein family (21).

| Name          | Complementation of growth<sup>a</sup> | PC-Lep processing<sup>b</sup> |
|---------------|--------------------------------------|------------------------------|
| S343–345      | –                                    | –                            |
| L343S         | +                                    | +                            |
| K345S         | +                                    | +                            |
| S348–348      | +                                    | +                            |
| S350–353      | +                                    | +                            |
| S354–356      | +                                    | +                            |
| S358–360      | +                                    | +                            |
| S354–360      | +                                    | +                            |
| S361–365      | –                                    | +/−                          |
| I361S         | –                                    | +/−                          |
| T362S         | +                                    | +                            |
| S363–365      | +                                    | +                            |
| S366–370      | +                                    | +                            |
| S370–372      | +                                    | +                            |

<sup>a</sup> The + complementation indicates that transformants were observed when using a plasmid encoding the YidC mutant to transform J87131 under glucose conditions. The – complementation indicates that the plasmid containing the YidC mutant did not transform the YidC depletion strain (see Experimental Procedures).

<sup>b</sup> + indicates >90% processing of PC-Lep; – indicates <20% processing of PC-Lep; and +/− indicates between 90 and 20% processing.

for the S343–345 mutant). To test whether there is a critical residue in regions 343–345 and 361–365, single serine mutants were constructed. We found that the I361S mutant was not functional, suggesting that the residue is important for YidC function. The lack of activity of the I361S mutant is not due to lack of expression of the protein or to protein instability, as the I361S protein was detectable on a Western blot (Fig. 7). In contrast, no essential residue was found in region 343–345, as all the single serine mutants were functional (Table I). Also, no essential residue was found in region 362–365, as the triple serine mutant S363–365 and the T362S mutant (Table I) functionally complemented the YidC depletion strain and promoted insertion of PC-Lep. Because we found that the isoleucine-to-serine substitution at position 361 inactivated YidC, we made further mutations to explore whether it is the hydrophobicity of the side chain that is important for YidC function. Indeed, we found that the I361V and I361L mutations were functional, whereas the I361A mutation was not, suggesting that a large aliphatic hydrophobic side chain (not a specific amino acid) is needed at this position for YidC activity. Taken together, the results indicate that the precise sequence of TM2 is not absolutely critical for YidC function.

Next, we found that the region near the center of the predicted third transmembrane region is important for YidC activity to promote the membrane insertion of the PC-Lep substrate. The serine mutants at positions 433, 434, 436, and 437 had impaired procoat processing (Table II), whereas most of the serine mutants with multiple serine residues introduced were functional. Interestingly, the F433S, L434S, and Y437S mutants had sufficient activity to complement the growth defect of the YidC depletion strain (Fig. 8A). However, the L436S mutant did not complement the YidC depletion strain. Most likely, the leucine at this position is important for protein stability, as the L436S mutant was poorly expressed (see Fig. 7). Because YidC is functional with an alanine at position 436, we believe that a hydrophobic side chain (not a specific amino acid residue) is needed at this position (Table II).

In TM6, only the absolutely conserved tyrosine residue (at position 516 of <i>E. coli</i> YidC) in the YidC/Oxa1/Alb3 family seems to be important for the membrane insertion activity of YidC (Table III). We found that substitution of the tyrosine with serine impaired PC-Lep processing (Fig. 8A and Table III). Nevertheless, this Y516S mutant had sufficient activity to complement the growth defect of the YidC depletion strain.

To test whether the six YidC single serine substitution mutants that were impaired in PC-Lep membrane insertion (S361S, F433S, L434S, L436S, Y437S, and Y516S) had sufficient activity to convert most of PC-Lep in the chase, we performed a pulse-chase experiment. In the pulse-chase experiments for F433S, L434S, and Y516S, PC-Lep processing was blocked to the same extent after 2 min (Fig. 8B), indicating that these mutants are quite defective in PC-Lep insertion. In contrast, PC-Lep processing was better for the I361S mutant, which did not complement the YidC depletion strain (Fig. 8B). This difference in the results between the membrane insertion and complementation assays may be due to the fact that the membrane insertion of essential membrane protein substrates that are required for cell growth was impaired with this mutant. On the other hand, because the F433S, L434S, and Y516S mutants, which were defective in PC-Lep insertion, could complement the YidC depletion strain, this suggests that these mutants have sufficient activity toward essential physiological substrates.

Another interesting observation we found from the serine scanning mutagenesis studies is that YidC could function in a number of cases when three to four hydrophilic serine residues were introduced at a time into TM2 and TM3 (see Tables I and...


**DISCUSSION**

*E. coli* YidC is an integral membrane protein that spans the membrane six times, with a large periplasmic domain between the first and second transmembrane domains (20). The first hydrophobic domain is an uncleaved signal sequence that functions to translocate the large periplasmic domain with the assistance of the SecYEG translocase (23, 24). Recently, we found that the first hydrophobic domain of YidC is not necessary for activity because YidC is functional when the cleavable signal peptide of the maltose-binding protein substitutes for the first hydrophobic domain of YidC (22).

In this study, we first made a number of YidC deletion mutants to examine which regions of YidC contribute to activity. We also tested whether the three consensus sequences found in TM2, TM3, TM5, and TM6 in the YidC/Oxa1/Alb3 family (21) are important for YidC function and also tested the importance of the conserved hydrophilic regions in the Oxa1 family of proteins located in loops C1 and P2 (Fig. 1) for YidC activity. These conserved hydrophilic regions have been proposed to play a role in substrate binding (21).

We found that the majority of the large 319-residue N-terminal periplasmic domain (loop P1) in the *E. coli* YidC protein is not necessary for the activity of YidC. For instance, 92% of this region could be deleted without impairing YidC function.
Interestingly, we isolated several YidC mutants that were fully functional in complementing the growth defect of the YidC depletion strain, but did not effectively stimulate membrane insertion of PC-Lep. These are the F433S, L434S, and Y516S mutants. It is not clear why these mutants behave in this way. One possibility is that they have sufficient activity to support cell growth despite impaired activity in promoting membrane insertion of PC-Lep and other membrane protein substrates. Another possibility is that the YidC mutants are still functional in the insertion of their essential physiological membrane protein substrates although impaired in membrane insertion of PC-Lep. In addition, we isolated a mutant (I361S) that did not complement the YidC depletion strain, but had better activity in stimulating PC-Lep membrane insertion than other mutants that complemented growth of the YidC depletion strain (Fig. 8A). In this case, YidC may be impaired in promoting membrane insertion of its physiological substrates that are essential for cell growth. These observations suggest that there are different YidC structural requirements for insertion of different membrane proteins, consistent with a recent report (25).

As far as we know, most membrane proteins that function as enzymes such as transporters or channel proteins almost always contain critical residues that form part of the active site. In these membrane proteins, the active-site residues promote catalysis and are absolutely conserved in their respective protein families. However, we were very surprised that the E. coli YidC protein does not appear to contain an absolutely essential residue. Even in the conserved five C-terminal transmembrane segments, YidC could tolerate dramatic sequence changes (Fig. 9). Although six YidC mutants were obtained that had impaired activity to promote membrane insertion of PC-Lep, in all cases, the residues that were substituted were not absolutely essential. Our hypothesis is that YidC promotes membrane insertion by binding its hydrophobic membrane-embedded parts to the hydrophobic stretches of the substrate that partitions into the membrane. The five C-terminal transmembrane regions of YidC function as a scaffold or platform that would provide an assembly site for hydrophobic regions of membrane proteins to insert into the membrane. This would not require catalytic residues. Because YidC seems to recognize the hydrophobic regions of the inserting substrate (3, 9) that will become the membrane-spanning regions in the inserted/assembled membrane protein, it is reasonable to suspect that the hydrophobic parts of YidC (not the hydrophilic regions) would be involved in this reaction, and this is what we observed. We identified six YidC mutants (I361S, F433S, L434S, L436S, Y437S, and Y516S) that had impaired activity. The residues at these positions all have either a large hydrophobic side chain or a slightly hydrophobic aromatic residue. The I361S and L436S mutants could not complement the YidC depletion strain. However, the amino acids at these two positions could be replaced by other residues without affecting function. Even more striking is the fact that TM4 and TM5 of YidC could be replaced by the unrelated transmembrane segments could be predicted to be quite short, such as those homologs found in Mycoplasma genitalium (51 residues), and Mycobacterium leprae (7 residues). In addition, cytoplasmic loop C1 and cytoplasmic loop C2 and the C-terminal tail of YidC (Fig. 1) are not important for function, as these regions could be deleted without affecting activity. Finally, alanine scanning mutagenesis indicated the short periplasmic loops (loops P2 and P3) do not have essential residues (Fig. 5).

Our work reported here proves that the five C-terminal transmembrane segments of YidC are important for activity (Fig. 6). The importance of these transmembrane segments is consistent with the observation that almost all members of the Oxa1 family have five N-terminal luminal domains that are predicted to be quite short, such as those homologs found in Mycoplasma genitalium (51 residues), and Mycobacterium leprae (7 residues). In addition, cytoplasmic loop C1 and cytoplasmic loop C2 and the C-terminal tail of YidC (Fig. 1) are not important for function, as these regions could be deleted without affecting activity. Finally, alanine scanning mutagenesis indicated the short periplasmic loops (loops P2 and P3) do not have essential residues (Fig. 5).

The role of the five transmembrane segments in YidC activity is not exactly known. They may function as a scaffold or platform that is necessary for YidC to carry out its biological function. One possibility is that the five transmembrane segments provide a structure that is essential for YidC to act as a membrane chaperone assisting in the transmembrane folding of hydrophobic domains into the lipid bilayer of both Sec-dependent and Sec-independent membrane proteins. Alternatively, the C-terminal transmembrane region is a structural element possibly allowing YidC to form a hydrophobic channel that promotes the insertion of membrane proteins into the membrane.
including the large periplasmic N-terminal domain and the conserved hydrophilic regions located in cytoplasmic loop C1 (21) and periplasmic loop P2 of the E. coli YidC protein do not appear to be of functional importance. Future studies will extend this study by investigating the affects of the YidC mutations on the insertion of Sec-dependent leader peptidase and the Sec-independent Pf3 coat protein substrates. These studies may provide significant insights to the YidC domains and their functions in membrane protein biogenesis.

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