YidC Is Involved in the Biogenesis of Anaerobic Respiratory Complexes in the Inner Membrane of *Escherichia coli*  
Claire E. Price and Arnold J. M. Driessen  

YidC of *Escherichia coli* belongs to the evolutionarily conserved Oxa1/Alb3/YidC family. Members of this family have all been implicated in membrane protein biogenesis of aerobic respiratory and energy-transducing proteins. YidC is essential for the insertion of subunit c of the F$_1$F$_0$-ATP synthase and subunit a of cytochrome o oxidase. The aim of this study was to investigate whether YidC plays a role during anaerobic growth of *Escherichia coli*, specifically when either nitrate or fumarate are used as terminal electron acceptors or under fermentative conditions. The effect of YidC depletion on the growth, enzyme activities, and protein levels in the inner membrane was determined. YidC is essential for all anaerobic growth conditions tested, and this is not because of the decreased levels of F$_1$F$_0$-ATP synthase in the inner membrane only. The results suggest a role for YidC in the membrane biogenesis of integral membrane parts of the anaerobic respiratory chain.

In *Escherichia coli* about 30% of all encoded proteins are located in the inner and outer membranes. The cytoplasmic or inner membrane contains essential energy-transducing complexes such as components of the electron transport chain, as well as solute transporters. Most integral inner membrane proteins are inserted co-translationally via the general secretory pathway otherwise known as the Sec system. In this pathway ribosome-bound nascent chains are targeted by the bacterial signal recognition particle to the SecYEG translocase via the signal recognition particle receptor FtsY. The membrane insertion of these proteins proceeds by a co-translational “threading mechanism” (1) in which the accessory protein YidC might play an important role in the clearance of transmembrane segments (TMSs)$^2$ from the SecYEG channel (2). A small subset of integral membrane proteins are targeted directly to YidC where they are integrated into the membrane in a Sec-independent manner. YidC belongs to the evolutionarily conserved Oxa1/Alb3/YidC family. Oxa1 (oxidase assembly) from yeast was the first member of this family to be described. It was originally identified as an essential factor for the biogenesis of respiratory complexes in the mitochondrion (3, 4), more specifically the insertion of subunits of the cytochrome bc$_1$ oxidase and ATP synthase. Alb3 is located in the thylakoid membranes of *Arabidopsis* chloroplasts (5) and is involved in the biogenesis of light-harvesting complexes. Members of the family have all been implicated in membrane protein biogenesis of respiratory and energy-transducing proteins. In *E. coli* it has been shown that YidC is essential for the insertion of subunit c of the F$_1$F$_0$-ATP synthase (F$_{1c}$) (6) and subunit a of cytochrome o oxidase (CyOA) (7, 8).

Although the effect of YidC depletion on the insertion of respiratory proteins has been studied in aerobically grown *E. coli* cells, the same has not been done for anaerobically grown cells. In the absence of oxygen, *E. coli* is able to use other organic (fermentation) and inorganic (anaerobic respiration) molecules as terminal electron acceptors. Nitrate is commonly used as an alternative electron acceptor to oxygen, and the protein complex nitrate reductase (NarGH) replaces the cytochrome o oxidase. The α and β subunits, NarG and NarH, respectively, are located in the cytoplasm and attach to the membrane via the γ subunit NarI to form the nitrate reductase complex (9). *E. coli* can also use fumarate as a terminal electron acceptor. The structure of fumarate reductase (FrdABCD) is similar to that of nitrate reductase except that the integral membrane part consists of two smaller subunits, namely FrdC and -D (10). Although another translocation pathway, the Tat system (Twin Arginine Translocation), has been shown to target the peripheral αβ subcomplex to the integral membrane γ subunit of some anaerobic respiratory complexes, for example the DMSO reductase (DmsABC) (11), this is not the case for all terminal reductases such as the fumarate reductase (11). Also, how the integral membrane γ subunits of these complexes are inserted into the membrane is not known.

In *E. coli* only three natural substrates of YidC have been identified, namely F$_{1c}$, CyOA, and more recently the ion channel MscL (12). The identification of additional inner membrane proteins, which rely solely on YidC for their insertion, would give insight into the insertion mechanism of YidC and a possible evolutionarily conserved function of the Oxa1/Alb3/YidC family. The aim of this study was to investigate whether YidC plays a role in the membrane biogenesis of anaerobic respiratory complexes, *i.e.* the complexes essential for growth when either nitrate or fumarate is used as a terminal electron ac-
YidC Function

ceptor or under fermentative conditions. Our data demonstrate that YidC is essential for all growth conditions tested and that this is not only because of decreased levels of F1F0-ATP synthase in the inner membrane. The results suggest a role for YidC in the membrane biogenesis of integral membrane subunits of the anaerobic respiratory chain.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The YidC depletion strain E. coli FTL10 (MC4100-A, ΔyidC, attB:(araC+, Pbad yidC+)) KanR (13) was a generous gift of Frank Sargent (University of East Anglia, Norwich, UK). Strains E. coli SF100 (F lacX74 galE galK thi rpsL (strA) ΔphoA(pvull), ΔompT) and NN100 (SF100, lpp D(uncB-C) zid::Tn10) (14) were used to test dependence of anaerobically grown cells on the F1F0-ATPase. Plasmid pTrc99A (15) was used to construct plasmid pET589 containing E. coli yidC (16). E. coli FTL10 was transformed with plasmids pTrc99A and pET589 to construct “YidC+” and “YidC−” depletion strains when grown on glucose-containing media.

Materials—Potassium nitrate was purchased from Fluka, and zinc powder, lapachol, and sodium fumarate were from Sigma. Texas Red was purchased from Invitrogen. Antiserum against YidC was raised in rabbits against purified His-tagged YidC (Agriserba AB, Sweden). Antiserum against fumarate reductase subunits FruA and FrdB, the nitrato reductase complex (NarGHI), subunit c of the F1F0-ATP synthase (F1c), leader peptidase (LepB), phage shock protein A (PspA), and subunit K of the NADH dehydrogenase 1 (NuoK) were generous gifts from Joel Weiner (University of Alberta, Canada), Axel Magalon (LCB-IBSM CNRS, Marseille, France), Gabriele Deckers-Hebestreit (University of Osnabrück, Germany), Jan Tommasen (Utrecht University, The Netherlands), William Wickner (Dartmouth Medical School, Hanover, NH), and Takao Yagi (The Scripps Research Institute, La Jolla, CA), respectively. The d-glucose HK assay kit was purchased from Megazyme and the nitrate test from Merck. Restore™ Western blot stripping buffer was purchased from Pierce. Alkaline phosphatase-conjugated anti-chicken, anti-mouse, and anti-rabbit IgG were purchased from Sigma.

Bacterial Growth and Processing—Cells were grown anaerobically at 37 °C on basal anaerobic growth medium supplemented with 0.1% yeast extract (Difco) as described previously (17). The following carbon sources, electron acceptors, and trace metals were added as indicated: 0.5% (w/v) glucose, 0.5% (v/v) glycerol, 1% (w/v) potassium nitrate, 10 mM sodium fumarate, 1 μM sodium selenite, and 1 μM sodium molybdate. Sodium selenite and sodium molybdate were only added when trace metals were added as indicated: 0.5% (w/v) glucose, 0.5% sodium selenite, and 1% (w/v) potassium nitrate was used as an electron acceptor. Sodium molybdate was added as a molybdate salts mixture. The glucose and nitrate contents of the growth media were measured spectrophotometrically at 25 °C using a Hitachi U-1100 spectrophotometer. The activities were measured by following the oxidation of reduced lapachol at 481 nm in the presence of nitrate or fumarate (22). The reactions were performed in an anaerobic cuvette with a rubber stopper under nitrogen. The extinction coefficient of lapachol was taken to be 2.66 mmol−1 cm−1, and specific activity was expressed as nanomoles of nitrate or fumarate reduced per min/mg of IMV.

Enzyme Assays—ATPase activity of IMVs was measured at 4 °C using malachite green as described (21). Nitrate reductase and fumarate reductase activities of IMVs were measured spectrophotometrically at 25 °C using a Hitachi U-1100 spectrophotometer. The activities were measured by following the oxidation of reduced lapachol at 481 nm in the presence of nitrate or fumarate (22). The reactions were performed in an anaerobic cuvette with a rubber stopper under nitrogen. The extinction coefficient of lapachol was taken to be 2.66 mmol−1 cm−1, and specific activity was expressed as nanomoles of nitrate or fumarate reduced per min/mg of IMV.

Translocation Assay—The presence of a functional Sec translocase was verified with IMVs using Texas Red-labeled proOmpA as a substrate (23). Proteinase K was added after the translocation reaction to digest any untranslocated material.

RESULTS

Depletion of YidC Leads to the Cessation of Growth under Anaerobic Conditions—To examine if YidC is needed for protein biogenesis of respiratory and energy transduction proteins expressed under anaerobic conditions, we examined the growth of E. coli FTL10 (13) under anaerobic conditions in the presence of various electron acceptors and carbon sources. Cells harboring either pET589 (YidC+) or pTrc99A (YidC−) were grown as described under “Experimental Procedures” until the cessation of the YidC− strain (adapted from Ref. 28). The following combinations of electron acceptors and carbon sources were used: glucose only; nitrate and glycerol; nitrate and glucose; fumarate and glycerol; fumarate and glucose. Under YidC−-depleting conditions, the YidC− strain ceased to grow under all conditions tested (Table 1). An aliquot of growth medium was sampled once cells had reached an absorbance of 0.6 after the cells had been diluted once, cleared of cells by
centrifugation, and assayed for D-glucose and nitrate. This confirmed that the cells were utilizing the glucose and nitrate in the growth medium (data not shown).

**Inner Membrane Protein Profile Is Largely Unchanged following YidC Depletion**—Examination of the inner membrane protein profile revealed that few changes occurred upon YidC depletion (supplemental Fig. 1A). The levels of the inner membrane protein leader peptidase (LepB), which is not strictly dependent on YidC for its insertion, are unaffected by YidC depletion as examined by immunoblot analysis (supplemental Fig. 1B). The levels of YidC were monitored by immunoblot analysis using antibodies against YidC. The intensity of the bands present on the immunoblots against YidC were quantified, and the blots were stripped and reprobed with antibodies against LepB, which was used as a control membrane protein. The intensity of the bands of YidC were then expressed as the ratio of the YidC and the LepB bands to compare relative levels of YidC in the membrane (Fig. 1A). This process was repeated for all immunoblots in the study. This showed that the levels of YidC in IMVs isolated from strains grown in the same growth medium for a comparable amount of time decrease substantially in the YidC–/H11002 strains.

**Sec Translocon Is Not Affected by YidC Depletion in E. coli FTL10**—There are many reports in the literature that show that YidC depletion does not affect the translocation of proteins via the Sec translocon or the Tat pathway (13, 24, 25). Using IMVs isolated from anaerobically glucose-grown cells, the translocation of Texas Red-labeled proOmpA still occurred in YidC–/H11002 IMVs (supplemental Fig. 1C). The processing of proOmpA is reduced, however, which can mostly be attributed to a disturbance of the proton motive force (PMF) because a similar effect is seen when the ionophores nigericin and valinomycin are added. No protection of Texas Red-labeled proOmpA from the externally added protease was observed when ATP is absent. These data confirm for anaerobically grown cells that the YidC– depletion does not affect the levels of the functional Sec translocon.

**Depletion of YidC Interferes with the Membrane Assembly of Fc—**Subunit c of the Fc is a well studied substrate of YidC. The levels of Fc in IMVs were found to decrease upon YidC depletion under anaerobic growth conditions tested (Fig. 1B). The total ATPase activities of IMVs were tested to confirm that the F1F0-ATP synthase was indeed not fully functional. It has been shown that 95% of the total ATPase activity of wild type IMVs can be attributed to the F1F0-ATP synthase.3 Under YidC-depleting conditions, the ATPase activity of YidC– IMVs isolated from cells under all growth conditions is reduced with respect to the YidC+ IMVs (Fig. 1C). The reduction in ATPase activity after YidC depletion was greater among cells grown in combination with nitrate or fumarate and glucose than those where glycerol was the carbon source.

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**TABLE 1**

Growth media used and stage at which the growth of the YidC– strain ceased

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Dilutions before growth ceased</th>
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<tbody>
<tr>
<td>Carbon source</td>
<td>Electron acceptor</td>
</tr>
<tr>
<td>Glucose</td>
<td>Nitrate</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Nitrate</td>
</tr>
<tr>
<td>Glucose</td>
<td>Nitrate</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Fumarate</td>
</tr>
<tr>
<td>Glucose</td>
<td>Fumarate</td>
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</table>

*YidC– cells were grown anaerobically in the presence of the indicated carbon source and electron acceptor to an A660 of 0.6 and diluted 2-fold with fresh medium, and the procedure was repeated until the cessation of the YidC– strain as described under “Experimental Procedures.”

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3 M. van de Laan, unpublished data.
The presence of the F1F0-ATP synthase is therefore essential to grow anaerobically in all other growth medium tested. Only glucose was severely impaired. These cells were, however, expected, the growth of NN100 cells in medium containing nitrate as the electron acceptor of NN100 reached a higher absorbance after 8 hours of growth (Fig. 3). As assayed by the oxidation of reduced lapachol was found to decrease upon YidC depletion (Fig. 4B). The data show that the levels of FrdA and FrdB decrease upon YidC depletion. Also, fumarate reductase activity of IMVs as assayed by the oxidation of reduced lapachol was found to decrease upon YidC depletion (Table 2). As with nitrate reductase, the activity of fumarate reductase was higher in IMVs isolated from fumarate/glycerol-grown cells in comparison with fumarate/glucose-grown cells.

Activity and Protein Levels of Fumarate Reductase in Membranes Decreases upon YidC Depletion—Because the decreased levels of functional F1F0-ATP synthase in the membrane are not responsible for the cessation of growth observed, the depletion of YidC must affect the assembly of some other membrane-located complex(es) needed for anaerobic respiration when nitrate or fumarate are the electron acceptors, and/or other essential membrane-localized functions. We therefore investigated the protein levels and enzyme activities of other membrane-located complexes essential for growth when nitrate or fumarate are the electron acceptors, namely the nitrate and fumarate reductases and the NADH dehydrogenase I. In the absence of oxygen, E. coli can use alternative electron acceptors to form an electron transport chain. In the presence of selenium, molybdenum, and high levels of nitrate, the expression of the nitrate reductase containing operon narGHIJ is induced rather than napFDAGHBC (26). Using an antibody against the entire complex, immunoblot analysis revealed that the levels of NarGHI decrease upon YidC depletion (Fig. 4A). The activity of the nitrate reductase was also assayed by the oxidation of a hydroxylated naphthoquinol menaquinol analogue, reduced lapachol, under anaerobic conditions. Under YidC-depleting conditions the reduction of nitrate by IMVs is reduced (Table 2). The activity of nitrate reductase was higher in IMVs isolated from nitrate/glycerol-grown cells in comparison with nitrate/glucose-grown cells. For nitrate/glycerol-grown cells, nitrate reductase activity was 2.4-fold reduced in the YidC- strain, and for nitrate/glucose-grown cells the reduction was greater, 3.5-fold.

Activity and Protein Levels of Fumarate Reductase in Membranes Decreases upon YidC Depletion—Using the same strategy as for the nitrate reductase complex, the effect of YidC depletion on another membrane-located terminal reductase, fumarate reductase, was examined. Immunoblot analysis of IMVs was carried out using antibodies directed against the α and β subunits of the reductase (FrdA and -B, respectively) (Fig. 4B). The data show that the levels of FrdA and FrdB decrease upon YidC depletion. Also, fumarate reductase activity of IMVs as assayed by the oxidation of reduced lapachol was found to decrease upon YidC depletion (Table 2). As with nitrate reductase, the activity of fumarate reductase was higher in IMVs isolated from fumarate/glycerol-grown cells in comparison with...
YidC Function

The data presented in this study demonstrate that under YidC-depleting conditions, E. coli is unable to grow anaerobically with either nitrate or fumarate as electron acceptors. The levels of various essential protein complexes were examined to determine possible reasons for the cessation of growth. Although some changes in the membrane protein profile were observed, the overall profile remained mostly unchanged under YidC-depleting conditions confirming the notion that most membrane proteins insert independently of YidC. It has been previously shown that neither the Sec translocase nor the Tat complex is influenced by YidC depletion in E. coli FTL10 (13, 25). One report suggested that SecE is weakly dependent on YidC (24), but the translocation of secretory proteins is only mildly affected by YidC depletion in aerobically grown cells (2).

Under our test conditions of anaerobic growth, the Sec translocon substrate, are unaffected by YidC depletion. Translocation was only marginally affected, which can mostly be attributed to a reduced capacity of YidC membrane to generate a PMF. In anaerobically glucose-grown (fermenting) cells, fumarate reductase activity was 1.7-fold reduced in the fumarate/glucose-grown cells. For fumarate/glucose-grown cells, fumarate reductase activity was 1.7-fold reduced in the YidC strain, and for fumarate/glucose-grown cells, the reduction was greater, 2.8-fold.

Protein Levels of the NADH Dehydrogenase I Subunit K—A knockdown of the human Oxa1l in HEK293 cells results in a reduced activity and protein content of the F_{1}F_{0}-ATP synthase and complex I, the NADH:ubiquinone oxidoreductase (27). E. coli expresses two types of NADH dehydrogenases. NADH dehydrogenase I bears more similarity to human complex I than NADH dehydrogenase II. Because of its small size (10.8 kDa), subunit K (NuoK), a subunit with three TMSs, is a possible YidC substrate. Immunoblot analysis of NuoK levels in IMVs isolated from nitrate/glucose-grown cells was carried out using antibodies directed against NuoK (Fig. 4C). The data show that the levels of NuoK decrease upon YidC depletion.

**DISCUSSION**

The data presented in this study demonstrate that under YidC-depleting conditions, E. coli is unable to grow anaerobically with either nitrate or fumarate as electron acceptors. The levels of various essential protein complexes were examined to determine possible reasons for the cessation of growth. Although some changes in the membrane protein profile were observed, the overall profile remained mostly unchanged under YidC-depleting conditions confirming the notion that most membrane proteins insert independently of YidC. It has been previously shown that neither the Sec translocase nor the Tat complex is influenced by YidC depletion in E. coli FTL10 (13, 25). One report suggested that SecE is weakly dependent on YidC (24), but the translocation of secretory proteins is only mildly affected by YidC depletion in aerobically grown cells (2).

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**TABLE 2**

Activity of the nitrate and fumarate reductases as assayed by lapachol oxidation of IMVs in the presence of electron acceptors 

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<thead>
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<th>Electron acceptor</th>
<th>Carbon source</th>
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<tr>
<td>Nitrate</td>
<td>Glycerol</td>
<td>+ 127.3 ± 33.6</td>
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<td></td>
<td>Glucose</td>
<td>- 51.9 ± 9.5</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Glucose</td>
<td>+ 81.2 ± 25.5</td>
</tr>
<tr>
<td>Fumarate</td>
<td>Glycerol</td>
<td>+ 15.2 ± 0.5</td>
</tr>
<tr>
<td>Fumarate</td>
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* Enzyme activities are expressed as nanomoles of nitrate or fumarate reduced per min per mg of IMV. Results are the mean of three separate measurements with indicated means ± S.E.

**FIGURE 4.** Depletion of YidC leads to a decrease in the amount of protein complexes essential for anaerobic respiration. A, immunoblot analysis of NarGHI complex levels in IMVs isolated from nitrate/glucose-grown cells. Levels of NarG, -H, and -I are expressed relative to levels of LepB. B, immunoblot analysis of FrdA (left panel) and FrdB (right panel) levels in IMVs. Levels of FrdA and FrdB are expressed relative to levels of LepB. C, immunoblot analysis of NuoK levels in IMVs. Levels of NuoK are expressed relative to levels of LepB.

Fumarate/glucose-grown cells. For fumarate/glucose-grown cells, fumarate reductase activity was 1.7-fold reduced in the YidC strain, and for fumarate/glucose-grown cells, the reduction was greater, 2.8-fold.

**Protein Levels of the NADH Dehydrogenase I Subunit K**—A knockdown of the human Oxa1l in HEK293 cells results in a reduced activity and protein content of the F_{1}F_{0}-ATP synthase and complex I, the NADH:ubiquinone oxidoreductase (27). E. coli expresses two types of NADH dehydrogenases. NADH dehydrogenase I consists of 13 subunits located at the inner membrane, encoded by the genes ndh. Seven of the subunits are integral membrane proteins with the number of TMSs ranging from 3 to 14. The membrane-located protein NADH dehydrogenase II encoded by the gene ndh is 47 kDa, and YidC depletion under aerobic conditions has no effect on its activity (25). Under anaerobic conditions in the presence of nitrate, both types can participate in respiration (28), and therefore NADH dehydrogenase activity assays cannot be used as a measure for the YidC depletion effect on these complexes. NADH dehydrogenase I is expressed under all anaerobic conditions (28), but is important only during growth in the presence of fumarate (28). NADH dehydrogenase I bears more similarity to human complex I than NADH dehydrogenase II. Because of its small size (10.8 kDa), subunit K (NuoK), a subunit with three TMSs, is a possible YidC substrate. Immunoblot analysis of NuoK levels in IMVs. Levels of NuoK are expressed relative to levels of LepB.

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YidC Function

Because YidC is essential for the insertion of Fo and CyoA, YidC depletion under aerobic growth conditions results in a massive up-regulation of PspA (25). PspA is up-regulated in response to stress conditions that result in the dissipation of the PMF (30). Research in this area has been largely focused on aerobically grown E. coli cells, but one proteomic study has shown that under osmotic stress the PspA protein is not induced when cells are grown anaerobically (31). Our data are in agreement with this study as the induction of PspA under the anaerobic growth conditions tested is greatly reduced when compared with aerobically grown cells. There are conflicting reports as to the mechanism by which the proteins contained in the psp operon enable the cell to cope with the dissipation of the PMF. One study in E. coli suggested that PspA and PspG switch the cell to anaerobic respiration and fermentation, thereby adjusting the energy usage and PMF generation of the cell (32). However, other microarray studies on Yersinia enterocolitica and E. coli showed only an up-regulation of the genes contained in the psp operon upon secretin overproduction and did not reveal a switch to anaerobic metabolism related to the induction of the Psp operon (33, 34). The mechanism by which Psp proteins operate is therefore still largely unclear under aerobic as well as anaerobic growth conditions.

Under anaerobic conditions and in the presence of nitrate, electron transfer from formate (from formate dehydrogenase) to nitrate is coupled to the generation of a PMF (9, 35). With fumarate reduction, the fumarate reductase is directly responsible for proton translocation (10). A reduction of the levels of fumarate and nitrate reductases and of the F1F0-ATP synthase in the YidC depletion strain will result both in a loss of the PMF and in PMF-dependent ATP synthesis. Because these anaerobically grown cells can still generate ATP by the metabolism of glucose or glycerol at a substrate level (36, 37), the F1F0-ATP synthase is not essential for ATP synthesis under these anaerobically respiring conditions. Indeed, the unc- strain E. coli NN100 was able to grow with either nitrate or fumarate as an electron acceptor.

It was shown that the protein levels and reducing activities of both the nitrate and the fumarate reductases were diminished upon YidC depletion. This could be responsible for the growth cessation observed. The α and β subunits of the nitrate reductase, NarG and NarH, respectively, are cytoplasmically located and attach to the integral membrane γ subunit NarI to form the nitrate reductase complex (9). Neither NarG, -H, or -I have any predicted Tat targeting sequences or signal peptide cleavage sites (TATFIND 1.4 (38) SignalP 3.0 (39)). NarG has, however, a vestige motif at its N terminus that appears to be a remnant of the Tat signature (40). It has been suggested that NarI, which is contained in the same operon as NarGHI, is a Tat-dependent chaperone that delivers the NarG and NarH complex to the membrane-inserted NarI (41). In the same study it was reported that the growth rate of cells in nitrate-containing media was impaired in the Δtat E. coli strain. This, however, conflicts with a previous study that reported that anaerobic growth with nitrate was not impaired in a ΔtatABCD E. coli strain (11). Neither studies determined whether mislocalization of the Tat-dependent formate dehydrogenase would have any effect on growth in nitrate-containing medium.

The Tat pathway has been implicated in the membrane location of anaerobic respiratory complexes, which have an αβγ structure but where the αβ subunits are periplasmically located (11, 42), for example, the NiFe hydrogenase-2 isoenzyme. The β subunit of this enzyme has one TMS, which is inserted by the Tat translocase itself and which is YidC independent (13, 43). The αβ subunits still attach and retain activity in the absence of the integral membrane γ subunit because of this C-terminally located TMS of the β subunit (43). In the nitrate reductase neither NarG nor NarH has a TMS and therefore they may need NarI to functionally attach to the membrane.

Fumarate reductase also has an αβγ structure with FrdA and FrdB forming a cytoplasmically located αβ subcomplex that attaches to the membrane via the integral membrane proteins FrdC and -D. The decrease in the levels of FrdA and FrdB associated with the inner membrane is suggestive of a role for YidC in the insertion of FrdC and FrdD because FrdA and FrdB cannot assemble at the bacterial membrane without first having the correct assembly of integral membrane parts FrdC and FrdD (44). FrdC and FrdD are both small (15 and 13 kDa, respectively) and do not have a TMS. Instead, they have a membrane targeting and insertion system, which in E. coli is responsible for the insertion and assembly of complex II, the succinate:ubiquinone oxidoreductase whose structure is analogous to fumarate reductase (27).

The levels of NuoK of the NADH dehydrogenase I were also examined following YidC depletion. These too were shown to decrease in the YidC- strain. If NuoK levels were decreased because of YidC dependence for its insertion, it would have a detrimental NADH dehydrogenase activity as most of the active peripheral arm of the complex is also absent if the NuoK subunit is (46). This could contribute to or be responsible for the cessation of growth under YidC-depleting conditions in the presence of fumarate. Interestingly, a recent cross-linking study indicates an association between YidC and NuoCD (47), lending further support to the notion that NADH dehydrogenase I assembly may be YidC-dependent.

Although it has been shown in this study and by others that neither the Sec translocase nor the Tat complex is influenced by YidC depletion in E. coli FT10, we cannot exclude other effects of YidC depletion that may affect the membrane targeting and insertion of the systems analyzed in this study. The other identified YidC only substrate, MscL, is essential only under osmotic stress conditions. Obviously, a defect in the membrane assembly and folding of other membrane proteins, for instance the transport systems involved in the uptake of nitrate and fumarate, may further contribute to the growth defect in cells depleted from YidC (48). Future studies will focus on the insertion and assembly of the integral membrane components of the nitrate and fumarate reductase complexes as well as those of the NADH dehydrogenase I.
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Supplementary Fig. 1. The inner membrane profiles of the cells once growth ceased in the YidC- strain remain largely unchanged upon YidC depletion. Cells were grown anaerobically in basal anaerobic growth medium supplemented with 0.1% yeast extract to which combinations of carbon sources and electron acceptors were added. When cell cultures reached an OD of 0.6, they were diluted 2 fold in fresh medium. This process was repeated until growth of the YidC- strain ceased. (A) IMVs were prepared from these cultures and analyzed by SDS-PAGE. IMVs were analyzed by immunoblotting using (B) anti-LepB. (C) The Sec translocase remains intact under YidC-depleting conditions. Cells grown anaerobically in glucose were depleted of YidC and IMVs were isolated. Texas Red-labeled proOmpA was translocated into IMVs in the absence (lanes 2, 3, 6 and 7) and presence of nigericin/valinomycin (lanes 4 and 5) and in the presence (lanes 2-5) and absence of ATP (lanes 6 and 7). Proteinase K was added after the translocation reaction to digest any untranslocated material.
Supplementary Figure 1