

YidC family members are involved in the membrane insertion, lateral integration, folding, and assembly of membrane proteins

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Members of the YidC family exist in all three domains of life, where they control the assembly of a large variety of membrane protein complexes that function as transporters, energy devices, or sensor proteins. Recent studies in bacteria have shown that YidC functions on its own as a membrane protein insertase independent of the Sec protein-conducting channel. YidC can also assist in the lateral integration and folding of membrane proteins that insert into the membrane via the Sec pathway.

The membrane surrounding every cell is an intriguingly dynamic structure that harbors a variety of protein complexes involved in central cellular functions and in sensing crucial environmental information. Transport proteins are involved in the uptake and release of metabolites and ions, whereas sensor proteins bind extracellular factors and transmit signals that control intracellular components. In addition, bacterial and organellar membranes contain enzyme complexes involved in the conversion of energy. Metabolic redox potential is converted into a proton motive force by the NADH dehydrogenase and cytochrome oxidase complexes. Light energy is converted into proton motive force by light-harvesting and photosynthetic complexes in the membrane.

Because of the great number and diversity of protein complexes involved in energy conversion, it is critical that the membrane insertion and assembly of each of these complexes is precisely regulated. The most extensively studied membrane insertion pathway involves the Sec protein translocase complex that is present in the ER, in the archaeal and bacterial membranes. This complex is also required for the secretion of proteins synthesized in the cytoplasm. The translocase structure forms a transmembrane channel with a diameter of 5–8 Å that must enlarge in order to permit the transmembrane passage of an α -helical protein chain (Van den Berg et al., 2004). Although the structure provides key

insights into the nature of the Sec protein conducting channel, it is clear that other activities must be involved in regulating and clearing the channel.

Insertion of membrane proteins by the Sec translocase requires that each hydrophobic anchor region is sensed during transport, the further translocation of the protein is halted, and the anchor region is laterally released from the central channel region. Some hints on how the lateral release is accomplished came with the discovery of YidC (Stuart and Neupert, 2000). YidC is a newly defined integral membrane protein that plays a role in membrane protein insertion (Samuelson et al., 2000; Scotti et al., 2000). YidC contacts inserting membrane proteins but not secreted proteins (Beck et al., 2001; Urbanus et al., 2001). It is becoming clear that the YidC protein operates in the recognition of transmembrane domains during transport (Scotti et al., 2000) and is involved in the lateral integration of transmembrane domains into the lipid bilayer.

There are proteins that insert into the membrane independently of the Sec translocase complex. These include, particularly, some small bacterial proteins, thylakoid proteins, and the mitochondrion-encoded proteins. In bacteria and chloroplasts, several Sec-independent proteins are translocated by the Tat pathway (Berks et al., 2003). The Tat pathway is unusual in that it can translocate folded proteins across the membrane (for review see Robinson and Bolhuis, 2001). It is dedicated to the export of a few membrane-associated protein subunits in the bacterial periplasm and thylakoid lumen of chloroplasts and is not a membrane insertion pathway in a strict sense. For mitochondria, proteins must insert by another pathway because they do not contain Tat or Sec translocases (Glick and Von Heijne, 1996). In 1997, a novel insertion pathway that uses the Oxa1 protein was discovered for mitochondrial inner membrane proteins (He and Fox, 1997; Hell et al., 1997). This discovery was shortly followed by the identification of the chloroplast homologue *Arabidopsis thaliana* Alb3 (Sundberg et al., 1997) and the bacterial

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Abbreviations used in this paper: LHCP, light-harvesting complex; SRP, signal recognition particle.

homologue YidC, both of which were shown to mediate membrane insertion (Moore et al., 2000; Samuelson et al., 2000).

The Oxa1–Alb3–YidC system that operates in mitochondria, chloroplasts, and bacteria is novel in that it plays a role in the insertion and folding of membrane proteins and does not require the hydrolysis of nucleotide triphosphates. Discovering how the precise steps of the integration and folding subreactions are controlled is a challenge for future research. In this review, we will highlight the recent advances showing that YidC functions as a membrane protein insertase and is involved in the folding of membrane proteins.

Eukaryotic systems

The first paper on the mitochondrial insertion component Oxa1 was published by a group of researchers studying the cytochrome oxidase complex in *Saccharomyces cerevisiae* (Bonney et al., 1994). They discovered that the *oxa1* gene is essential for the assembly of the complex (Altamura et al., 1996). Mutants in the *oxa1* gene that affected cellular respiration were isolated. This gene encodes a protein, termed Oxa1, that has five membrane-spanning regions and a molecular mass of 36 kD. The nucleus-encoded proteins ATPase-su9, CoxII, and Oxa1 itself, were found to use the “conservative” pathway, which possesses features similar to the Sec-independent bacterial membrane insertion pathway (Hell et al., 1997). In this pathway (Fig. 1 A), the nucleus-encoded mitochondrial proteins are first translocated into the mitochondrial matrix from the cytoplasm and are subsequently inserted into the inner membrane. In addition, mitochondrion-encoded proteins such as Cox1p and Cox3p also use this Oxa1-dependent pathway (Hell et al., 2001). Interestingly, recent results show that Oxa1 interacts with the ribosome in mitochondria via its COOH-terminal tail, suggesting a cotranslational insertion mechanism for mitochondrion-encoded proteins (Jia et al., 2003; Szyrach et al., 2003).

Alb3, the homologue of Oxa1, is found in the thylakoid membranes of the chloroplast (Fig. 1 B). Alb3 spans the membrane five times and has a molecular mass of 40 kD. A mutant in the *alb3* gene was found to be deficient for the proper assembly of proteins in thylakoid membranes (Sundberg et al., 1997; Bellafiore et al., 2002). Moreover, efficient assembly of photosystem II in *Chlamydomonas reinhardtii* requires Alb3.1p, a homologue of *Arabidopsis* Alb3 (Ossenbuhl et al., 2004). In particular, the insertion and assembly of light-harvesting complexes (LHCPs) are catalyzed by Alb3 (Moore et al., 2000; Bellafiore et al., 2002). These proteins are first posttranslationally imported into the chloroplast from the cytoplasm; then, once in the stroma, they are targeted to the thylakoid by the SRP complex composed of cpSRP54 and the novel cpSRP43 component (Moore et al., 2000).

Recently, an Alb3 chloroplast inner membrane protein called ARTEMIS was shown to be a functional member of the YidC family (Funes et al., 2004). This protein can partially substitute for the yeast Oxa1 in the insertion and assembly of membrane proteins in mitochondria. The work suggests that there may be a novel YidC homologue involved in protein insertion in the chloroplast inner membrane.

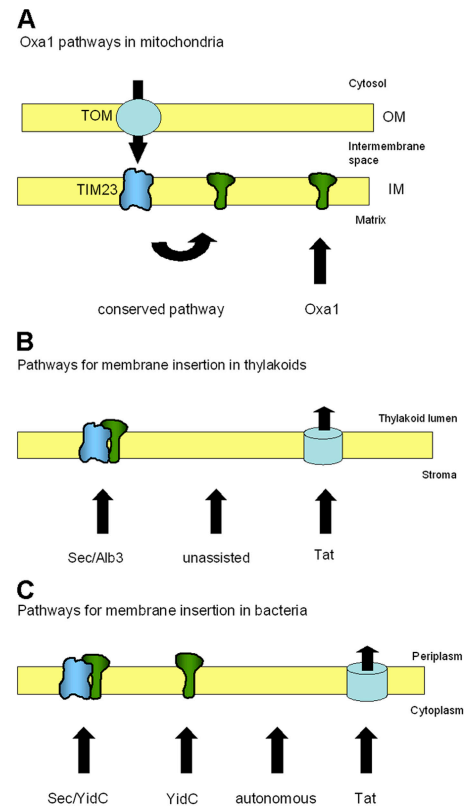


Figure 1. Pathways of mitochondrial, thylakoidal, and bacterial membrane insertion. (A) Nucleus-encoded mitochondrial proteins are translocated by the TOM and TIM complexes into the mitochondrial matrix, and then are inserted into the mitochondrial inner membrane involving the Oxa1 protein. Mitochondrion-encoded proteins directly access Oxa1 for their membrane insertion. (B) For proteins inserted into the thylakoid membrane, three distinct pathways are known. LHCP proteins use Alb3, possibly in conjunction with the Sec translocase. Proteins exported by the Tat pathway use the proton gradient; in contrast, other proteins, which are typically small, insert into the membrane by an unassisted pathway. (C) Insertion into the bacterial membrane uses four pathways. Most proteins use the pathway Sec–YidC. Some small proteins use the YidC pathway or an autonomous pathway. A limited number of metal-containing, membrane-associated periplasmic proteins use a pathway that is homologous to the Tat pathway of thylakoids.

Prokaryotic systems

To date, members of the YidC family have been found in all genomes of eubacteria and in very distantly related archaea. In *Escherichia coli*, the YidC protein is a six-membrane-spanning 61-kD protein. Compared with its homologues Oxa1 and Alb3, YidC has an additional transmembrane segment close to its NH₂ terminus and a large periplasmic domain of 320 amino acid residues between the first and second transmembrane regions. The protein is found primarily as a monomer, but some dimeric forms have been reported (van der Does et al., 2003). A molecular interaction with the Sec translocase has been found; this interaction is likely due to a direct binding of YidC to the accessory SecDF proteins (Nouwen and Driessen, 2002). YidC has been determined to be important for the insertion of Sec-independent proteins and the facilitation of Sec-dependent proteins (Fig. 1 C; Samuelson et al., 2000). For FtsQ, a single membrane-

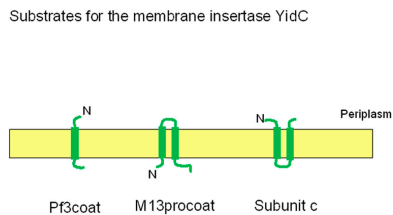


Figure 2. Substrates of the bacterial YidC pathway. Pf3 coat protein spans the membrane with an 18-residue NH₂-terminal domain in the periplasm and an 8-residue COOH-terminal domain in the cytoplasm. The M13 procoat protein spans the membrane twice and exposes a 20-residue hydrophilic loop in the periplasm. Subunit c of the F₁F₀ ATP synthase has an NH₂-terminal tail of approximately seven residues and a carboxyl-terminal tail of three residues, both localized on the periplasmic face of the membrane.

spanning protein of *E. coli*, photo cross-linking experiments suggested that this protein first contacts the Sec components, and then only at a later stage contacts YidC (Urbanus et al., 2001). For the mannitol permease MtlA, a five-membrane-spanning protein, contacts with YidC and SecY appeared almost simultaneously with nascent chains (Beck et al., 2001). This suggests that the Sec complex and YidC work together for the membrane insertion of these proteins.

Membrane proteins that use both the Sec translocase and YidC are usually targeted by a signal recognition particle (SRP). Prominent examples are the FtsQ and MtlA proteins (Koch et al., 1999; Scotti et al., 1999). However, SRP function is not limited to the targeting of proteins to the Sec translocase because a Sec-independent fusion protein that uses the YidC pathway also requires SRP for its targeting (Froderberg et al., 2003). It appears that YidC may form a complex with the SRP components Ffh and FtsY because the chloroplast homologue Alb3 has been found to be associated with a large complex that includes cpSRP, cpFtsY, and cpSecY (Moore et al., 2003), and because Alb3 can substitute for YidC in *E. coli* (Jiang et al., 2002).

Sec-independent proteins use a distinct pathway in which YidC can function on its own. These membrane proteins cannot be cross-linked to the Sec components and show contacts only to YidC (Chen et al., 2002). Because YidC in *E. coli* membranes is present in excess amounts over SecY, only a fraction of YidC can be bound to the Sec translocase.

Substrates of YidC

The YidC pathway mediates the insertion of a diverse group of Sec-independent substrates, including viral coat proteins and respiratory chain protein subunits (Fig. 2). One of the best studied Sec-independent proteins is the single membrane-spanning protein Pf3 coat (of 44 amino acid residues), the major envelope component of the Pf3 filamentous phage. After synthesis, the Pf3 coat is rapidly targeted to the membrane and partitions into the bilayer (Chen et al., 2002). Next, the transmembrane segment inserts across the membrane, and the 18-residue-long NH₂-terminal domain is transferred to the trans side of the membrane. This latter reaction is driven by both the electrochemical membrane potential and the YidC insertase. The YidC protein binds directly to the hydrophobic region of the Pf3 substrate protein as suggested by photo cross-linking studies (Chen et al., 2002). In-

triguingly, when the electrochemical potential is dissipated to block the Pf3 coat insertion step, the interaction between the Pf3 coat and YidC can still be observed. This unequivocally shows that YidC interacts with its substrate protein before moving the hydrophobic region into the membrane.

M13 procoat also requires YidC for its membrane insertion. M13 procoat is made in a precursor form with a signal peptide that is cleaved by signal peptidase to generate the single membrane-spanning coat protein. The precursor protein contains a 20-amino acid periplasmic loop that translocates only in the presence of YidC (Samuelson et al., 2000, 2001). Similar to the insertion of the Pf3 coat protein, the translocation of the M13 procoat loop depends on the electrochemical membrane potential (Zimmermann et al., 1982). However, M13 procoat protein mutants that insert across the membrane independent of the membrane potential still require YidC (Samuelson et al., 2001).

An endogenous substrate for YidC is the *E. coli* F₁F₀ ATP synthase subunit c. This substrate spans the membrane twice and has an inverted topology compared with that of the M13 procoat (Fig. 2). The two terminal tails of seven and three residues, respectively, have to be translocated to the periplasm. The translocation of these terminal tails was recently shown to require YidC (Yi et al., 2003), but neither the membrane potential, the Sec translocase, nor the SRP complex is involved in the membrane insertion process (van der Laan et al., 2004a).

The parts of YidC that are important for its function as a membrane insertase have been studied with a detailed collection of deletion and substitution mutants (Jiang et al., 2003). Transmembrane regions 2, 3, and 6 are important for activity because six single serine mutations that impaired membrane insertase activity were isolated within these hydrophobic regions. In contrast, transmembrane regions 4 and 5 do not appear to be as critical, because YidC is functional with unrelated hydrophobic stretches replacing transmembrane regions 4 and 5. Likewise, the large NH₂-terminal hydrophilic domain is not functionally involved; over 90% of this region can be deleted without an impact on the membrane insertase activity of YidC. These results suggest that YidC may function as a scaffold for the insertion and assembly of hydrophobic regions of membrane proteins.

YidC functions as a membrane insertase

Recently, it was unequivocally shown that YidC can function as an insertase, separate from the Sec translocase (Serek et al., 2004). In Serek et al. (2004), the membrane insertion of the purified Pf3 coat was reconstituted using YidC proteoliposomes. Microgram amounts of the Pf3 protein were inserted with this system, showing that YidC functions catalytically and is sufficient to support the reaction. The membrane insertion of the substrates is rapid, occurring in a few minutes.

YidC was also shown to play an exclusive role in the insertion of the F₁F₀ ATP synthase subunit c (van der Laan et al., 2004b). Subunit c inserted into lipid vesicles containing only YidC, but no insertion was observed when the lipid vesicles contained only SecYEG. In contrast with the coat proteins, subunit c does not require the electrochemical membrane potential for insertion and can only form an oli-

gomer if YidC is in the vesicles. Together, these studies established that YidC is sufficient for inserting a subset of membrane proteins and can operate on its own.

At the present time, nothing is known about the molecular mechanism of insertase function. These proteins might provide a platform for hydrophobic domains of nascent membrane proteins to straighten out and to orient α -helical domains perpendicular to the membrane, thereby achieving a transmembrane conformation. In this case, a simple binding and release mechanism of the membrane insertase with its substrate might be sufficient. Another possibility is that the membrane insertases themselves are dynamic proteins that allow the flip-flop of the membrane protein regions from the cis to the trans side, as was proposed for lipid transporters (van Helvoort et al., 1996; Raggers et al., 2000).

YidC is involved in the integration of Sec-dependent proteins

Besides acting as a catalyst for the insertion of membrane proteins from the cytoplasm, YidC is involved in the lateral integration of membrane proteins into the lipid bilayer after the membrane proteins have inserted into the Sec channel. This proposal was initially based on *in vitro* data showing that the hydrophobic domain of FtsQ first interacts with SecY, and then interacts with YidC. Another study extended this idea (Beck et al., 2001). Therefore, YidC might act as a membrane integrase working with the Sec translocase in transferring the hydrophobic region of the membrane protein from the Sec channel and allowing it to partition into the membrane bilayer.

Indeed, when some Sec-dependent proteins, such as leader peptidase or TatC, were overexpressed under YidC-depleted conditions, jamming of the translocase channel was observed (Samuelson et al., 2001; Yi et al., 2003). Because the block in protein export depended on the overexpression of the membrane protein, it was concluded that YidC is important for the removal of the overproduced membrane protein from the Sec channel (Samuelson et al., 2001). It is possible that for most membrane proteins, this integrase function is not essential. Using SecYEG proteoliposomes, it has been shown that FtsQ can stably integrate into the membrane in the absence of YidC (van der Laan et al., 2004b).

YidC is involved in the folding of membrane proteins

In addition, recent studies show that YidC plays an important role in the folding of membrane proteins. The first evidence of this came from photo cross-linking studies with the multispanning MtlA membrane protein. Beck et al. (2001) observed that the transmembrane segments of MtlA contacted YidC when the protein was still in the vicinity of SecE (Beck et al., 2001) and that these transmembrane segments remained in the vicinity of YidC even after it had left the Sec translocase. These observations suggest that YidC functions as a collection site for the transmembrane regions and may be important for a membrane protein to achieve its proper three-dimensional arrangement of transmembrane helices within the membrane.

X-ray structure studies show that multispanning membrane proteins are tightly folded, and the transmembrane helices are held together by hydrophobic interactions. These

structures generally contain no internal lipid molecules. Therefore, the folding process has to exclude lipid molecules. It has been proposed that the tight folding occurs in steps (Popot and Engelman, 1990). In this folding process, YidC might provide a protective environment that allows the tight folding of a membrane protein to occur by excluding the interference of lipid molecules.

Strikingly, it was recently reported, using conformational antibodies, that YidC might be involved in the folding of the membrane-inserted lactose permease LacY (Nagamori et al., 2004). The 12-membrane-spanning lactose transporter is inserted into the membrane by the Sec translocase (Ito and Akiyama, 1991). In the absence of YidC, the binding of an antibody that recognizes the periplasmic loop between the transmembrane regions 7 and 8 was inhibited. Similarly, an antibody that recognizes the two cytoplasmic loops of the transmembrane regions 8/9 and 10/11 only bound to the LacY protein when YidC was present (Nagamori et al., 2004). These findings suggest that YidC is important for the inserted membrane protein to achieve its correct tertiary structure.

Involvement of YidC in the assembly of membrane protein complexes

After the initial discovery of the YidC/Oxa/Alb3 family of proteins in 1994, it was proposed that these proteins play a role in the assembly of protein complexes (Bonnefoy et al., 1994). Indeed, the assembly of respiratory chain complexes, ATP synthase, and LHCPs may be facilitated by Oxa1 or YidC. In mitochondria, the oxidative respiratory chain consists of four membrane complexes, of which the terminal complex is the cytochrome *c* oxidase. The assembly of the cytochrome *c* oxidase complex is inhibited in *oxa1*-deficient cells, whereas complex III assembly in these cells seemed to be unaffected (Altamura et al., 1996).

In *E. coli*, the cytochrome bo_3 oxidase complex (Fig. 3) consists of four subunits with a total of 23 transmembrane helices within the complex (Abramson et al., 2000). It was reported that when YidC was depleted for 2.5 h, the O_2 consumption of the cells was greatly reduced, correlating with a decrease in the membrane content of subunit II of the oxidase (van der Laan et al., 2003). Therefore, the assembly of

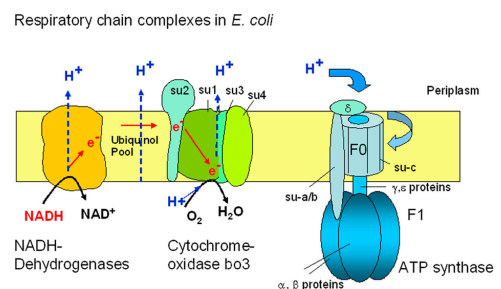


Figure 3. Respiratory chain complexes in *E. coli*. Under aerobic growth conditions, the respiratory chain consists of the NADH dehydrogenases and the cytochrome bo_3 oxidase complex. NADH dehydrogenase II is monomeric, whereas NADH dehydrogenase I has 13 subunits. Both dehydrogenases generate ubiquinol that is then oxidized by the cytochrome bo_3 complex of four subunits. The ATP synthase has the integral membrane component F_0 with the subunits a , b_2 , and c_{10} , and a peripheral F_1 component.

these complexes depends on YidC, or some protein subunits within the complex require YidC for membrane insertion, thereby preventing the assembly of the complex under the YidC-depleted conditions. Hence, YidC may be essential for bacteria because energy-transducing protein subunits are endogenous substrates (van der Laan et al., 2004a).

Assembly of the ATP synthase complex of *E. coli* (Fig. 3) most likely starts with subunit c. Subunit c, with two transmembrane segments, forms complexes of 10mers in the membrane. Subunit a requires c for stability (Hermolin and Fillingame, 1995), and is accompanied by subunit b to build the F₀ part of the synthase. The F₁ part of the ATP synthase is added on to the membrane-assembled F₀ complex from the cytoplasmic side. Because subunit c requires YidC for its membrane insertion, YidC affects the assembly process of the entire F₀ complex.

Conclusion

The recent findings show that YidC family members have multiple functions in membrane biogenesis. For Sec-independent membrane proteins, the bacterial YidC possesses catalytic activity as a membrane insertase and can operate on its own in a reconstituted system. The Sec-associated YidC may also facilitate the lateral integration of a substrate protein into the lipid bilayer after leaving the translocase. YidC probably facilitates the bundling of transmembrane domains and the folding of the membrane protein in this process. Most likely, the YidC protein provides a platform that allows the molecular interaction between several transmembrane regions of the inserted polytopic membrane protein that results in its three-dimensional structure.

The assembly of multimeric membrane protein complexes might involve YidC in a similar fashion, such that the transmembrane helices of the participating subunit proteins can correctly interact with each other. Future experiments will have to unravel the mechanistic details of how this occurs in membrane biogenesis.

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