YidC/Alb3/Oxa1 Family of Insertases*

Published, JBC Papers in Press, May 6, 2015, DOI 10.1074/jbc.R115.638171

Seth W. Hennon, Raunak Soman, Lu Zhu, and Ross E. Dalbey
From the Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio 43210

The YidC/Alb3/Oxa1 family functions in the insertion and folding of proteins in the bacterial cytoplasmic membrane, the chloroplast thylakoid membrane, and the mitochondrial inner membrane. All members share a conserved region composed of five transmembrane regions. These proteins mediate membrane insertion of an assorted group of proteins, ranging from respiratory subunits in the mitochondria and light-harvesting chlorophyll-binding proteins in chloroplasts to ATP synthase subunits in bacteria. This review discusses the YidC/Alb3/Oxa1 protein family as well as their function in membrane insertion and two new structures of the bacterial YidC, which suggest a mechanism for membrane insertion by this family of insertases.

In all cells, membrane proteins play crucial roles in energy production, substrate transport, signaling, and metabolite exchange. They function as ATPases, photosynthetic complexes, chemosensors, and permeases. Membrane proteins make up 25–30% of the proteins in a cell and comprise over 50% of known drug targets. To assemble proteins into the membrane lipid bilayer, translocation and insertion machineries are required in cells. The Sec translocase is the major translocase that inserts proteins into and across the endoplasmic reticulum of eukaryotic cells, the cytoplasmic membrane of bacterial and archaeal cells, and the thylakoid membrane of plants. When translocating proteins across the membrane, the Sec machinery does so in an unfolded state (1, 2). Also operating within the eukaryotic cells, the guided entry of tail-anchored (GET) machinery delivers tail-anchored proteins to the endoplasmic reticulum membrane for membrane insertion by a post-translational mechanism (3). A very different translocase called the twin arginine translocation (Tat) machinery functions to translocate folded proteins across the membrane (4, 5). The Tat pathway can translocate proteins across the cytoplasmic membrane in bacterial and archaeal cells and across the thylakoid membrane of chloroplasts.

This review will focus on the YidC/Oxa1/Alb3 family of proteins that operate in bacteria and certain eukaryotic organelles to facilitate membrane protein insertion. We will discuss the distribution and function of these insertases and highlight the recent structural work on these novel proteins, which provides insight into how these proteins catalyze membrane protein insertion and protein assembly at a molecular level.

Distribution, Topology, and Function of YidC/Oxa1/Alb3 Proteins

In eukaryotes, there are two members of the YidC/Oxa1/Alb3 family that span the membrane five times (6, 7): Oxa1 and Oxa2 (Cox18) in the mitochondrial inner membrane (8) and Alb3 and Alb4 in the chloroplast thylakoid membrane (9). The two paralogs in each system differ in their C-terminal region. The mitochondrial Oxa1 contains a long positively charged C-terminal region that constitutes the ribosomal binding domain, whereas the Oxa2 lacks this domain (8). Oxa1 functions in co-translational insertion, whereas Oxa2 functions in post-translational insertion (10–12). In the chloroplast, Alb3 also possesses a long C-terminal region, but it recognizes a special targeting protein called SRP43, whereas Alb4 lacks this C-terminal domain (13). In bacteria, the number of YidC paralogs and the number of membrane-spanning regions possessed by YidC can vary. In most Gram-positive bacteria, two paralogs (YidC1 and YidC2) can be found, and they both span the membrane five times (14, 15). YidC2 proteins possess a long C-terminal region that can directly bind to the ribosome, whereas YidC1 lacks such a domain (16). In Gram-negative bacteria, only one YidC is found, and it contains an extra TM2 segment at the N-terminus of the protein, which allows the protein to span the periplasmic domain between TM1 and TM2 (17). In some organisms, there can be multiple paralogs. For example, most plants have four YidC members: two paralogs in mitochondria and two in chloroplasts.

Interestingly, in Bacillus subtilis, the gene expression of a YidC paralog is regulated by a novel post-translational mechanism. Chiba et al. (18) discovered that when SpoIIIJ (YidC1) is functional, the expression of YqiG (YidC2) is repressed, whereas the expression of YqiG is up-regulated when SpoIIIJ is inactivated. Using a genetic approach, Chiba et al. (18) found that SpoIIIJ inserts the MifM sensor protein that is encoded by the mifM gene, which precedes the yqiG gene. When SpoIIIJ inserts MifM, the RNA hairpin loop following mifM masks the Shine-Dalgarno sequence required for expression of yqiG. However, when SpoIIIJ does not insert MifM, the membrane protein substrate undergoes translational arrest. This arrest causes changes in the RNA structure leading to unfolding of the hairpin loop and expression of YqiG. Arrest of protein synthesis occurs by a multisite ribosomal stalling mechanism (19) that is different from the stalling mechanism that is observed with SecM, which controls the expression of the secA gene in Escherichia coli (20).

The cellular function of the YidC family members is important for the assembly of energy-transducing complexes (21). The bacterial YidC and mitochondrial Oxa1 primarily function to insert and assemble the protein complexes involved with respiration (21–25), whereas the chloroplast Alb3 paralogs are necessary for photosynthesis and thylakoid biogenesis (26–29).

*This work was supported by National Science Foundation Grant MCB-1052033 (to R. E. D.)

1 To whom correspondence should be addressed. E-mail: dalbey@chemistry.ohio-state.edu.

2 The abbreviations used are: TM, transmembrane; LHCP, light-harvesting chlorophyll-binding protein; cpSRP, chloroplast SRP; SRP, signal recognition particle; HTL, holo-translocon; PSII, Photosystem II.
The enzyme activity of these insertases is remarkably conserved (11, 30–33) (Table 1). For example, the mitochondrial Oxa1 and bacterial YidC (with the C-terminal ribosome binding domain appended) can functionally substitute for each other (11, 33), and the chloroplast Arabidopsis thaliana Alb3 and Alb4 can replace the E. coli YidC and function to insert proteins into the bacterial cytoplasmic membrane (31, 32). Table 1 shows the substrates that have been identified for these insertases in mitochondria, chloroplast, and bacteria.

**Oxa1 Family**

The YidC/Oxa1/Alb3 family of proteins was discovered in 1994 by scientists in the mitochondrial field. The key findings were that a mutation in a new protein called Oxa1 (for oxidase assembly factor) affected cytochrome oxidase assembly (22, 23) and the formation of the F1F0-ATP synthase (24). The enzyme activity of these insertases is remarkably conserved (11, 30–33) (Table 1). For example, the mitochondrial Oxa1 and bacterial YidC (with the C-terminal ribosome binding domain appended) can functionally substitute for each other (11, 33), and the chloroplast Arabidopsis thaliana Alb3 and Alb4 can replace the E. coli YidC and function to insert proteins into the bacterial cytoplasmic membrane (31, 32). Table 1 shows the substrates that have been identified for these insertases in mitochondria, chloroplast, and bacteria.

**TABLE 1**

**Known substrates of each YidC homolog**
The YidC family is used mainly to insert proteins associated with respiration. *F*, non-native protein, *I*, aided by Oxa2.

| YidC Family | Function | Independent |
|-------------|----------|-------------|
| Foc         | F$_{1}$F$_{0}$ ATP synthase subunit |
| Mocl        | Large-conductance mechanosensitive channel |
| Ts1L        | Part of the type VI secretion system |
| M13 procoat $^*$ | M13 bacteriophage major coat protein |
| Pf3 coat $^*$ | Pf3 bacteriophage major coat protein |

| Oxa1 Family | Mitochondrial Encoded |
|-------------|-----------------------|
| Atp6        | F$_{1}$F$_{0}$ ATP synthase subunit |
| Atp9        | F$_{1}$F$_{0}$ ATP synthase subunit |
| Cox1        | Cytochrome c oxidase subunit |
| Cox2        | Cytochrome c oxidase subunit |
| Cox3        | Cytochrome c oxidase subunit |
| Cytb        | Cytochrome c oxidase subunit |

| Nuclear Encoded | Function |
|-----------------|----------|
| Oxa1            | Existing Oxa1 is required for new Oxa1 insertion |
| Md11            | ATP dependent permease |

**Cooperative with Sec | Function **

| Foa | F$_{1}$F$_{0}$ ATP synthase subunit |
| Fob | F$_{1}$F$_{0}$ ATP synthase subunit |
| NuoX | NADH-quinone oxidoreductase subunit |
| CyoA | Cytochrome b(3) ubiquinol oxidase subunit |

**Alb3 Family**

| Alb3 | Function |
|------|----------|
| LHCP | Organizes chlorophyll into chlorophyll protein complex CII |

| Alb4 | Function |
| CF0l | CF$_{1}$F$_{0}$ ATP synthase subunit |
| CF1b | CF$_{1}$F$_{0}$ ATP synthase subunit |

**MINIREVIEW: Membrane Protein Insertases**

of nuclear-encoded mitochondrial proteins. Hell et al. (37) showed that the nuclear encoded Oxa1 is involved in its own biogenesis. Oxa1 is synthesized in the cytosol and subsequently imported into the mitochondria where it assembles into the inner membrane. For this process, the Oxa1 machinery already residing in the inner membrane is critical for the translocation of the N-tail of the transported Oxa1, which is initially imported into the matrix (37). Oxa1 also participates in the membrane biogenesis of the nuclear encoded Md11, a six-transmembrane-spanning protein of the mitochondrial inner membrane with its N and C termini in the matrix (38). During the import of Md11 into the mitochondria, the TIM22 complex and Oxa1 machineries cooperate to insert the protein into the membrane. During this process, the TIM22 translocon engages the Md11 membrane protein at the inner membrane and integrates the N-terminal and C-terminal domains into the membrane by a stop transfer mechanism. Strikingly, the central region of Md11 is completely translocated into the mitochondrial matrix and then re-inserted into the inner membrane by Oxa1.

A distinctive feature of Oxa1 is that it contains a long positively charged C-terminal tail exposed to the mitochondrial matrix. This domain enables Oxa1 to be permanently bound to the matrix-localized ribosome (12, 39). The ribosome-bound Oxa1 is in proximity to the large ribosomal proteins Mrp20 (39) and Mrp40 (40), which are homologous to bacterial ribosomal proteins L23 and L34 and known to be located at the polypeptide exit site within the large ribosome subunit. Interestingly, deletions in the mitochondrial Mrb20 and Mrp40 proteins, which do not affect ribosomal protein synthesis, have profound effects on the assembly of the oxidative phosphorylation complexes in the inner membrane (40, 41). These and other studies highlight the importance of Oxa1-ribosome complexes for the assembly of respiratory chain complexes (40, 42).

Oxa2 (also called Cox18), the other Oxa1 paralog, is important for the assembly of cytochrome c oxidase and specifically for the biogenesis of Cox2, which is encoded in the mitochondrial genome and transfers the electron from cytochrome c to Cox1. Oxa2 facilitates the post-translational translocation of the C-terminal domain of Cox2 in both S. cerevisiae and Neurospora crassa (8, 43, 44). In the inner membrane, Oxa2 forms a complex with Pnt1 and Mss2 in which they cooperate together in the biogenesis of Cox2 (43). Interestingly, the translocation and assembly of Cox2 can occur in the absence of Oxa2; however, this requires Oxa1 to be overexpressed and the protein Yme1 (an ATP-dependent mitochondrial protease residing in the inner membrane that is also involved in protein folding) (45) to act as a chaperone to help fold and assemble Cox2, leading to a functional cytochrome c oxidase complex (46).

**Alb3 Family**

In chloroplasts, transposon tagging in Arabidopsis thaliana revealed that the ALB3 gene encodes a protein crucial for photosynthesis. Knockouts of the gene ALBINO3 (encoding Alb3) led to a photosynthesis-defective mutant with an Albino appearance (26), giving rise to its name Alb3.

Alb3 is critical for the post-translational insertion of the light-harvesting chlorophyll-binding protein (LHCP) into thy-
MINIREVIEW: Membrane Protein Insertases

YidC Family

Six years after Sundberg et al. (23) established that the yidC gene was present in the genome of both Gram-negative and Gram-positive bacteria, the role of YidC in membrane protein biogenesis was discovered. Scotti et al. (58) showed that YidC can be cross-linked to newly synthesized FtsQ during membrane protein insertion and that YidC co-purifies with SecYEG and SecDFYajC. Furthermore, YidC was discovered to be essential for the growth of E. coli and to promote the insertion of the Sec-independent M13 phage procoat protein (59), which was previously thought to be inserted by an unassisted mechanism.

The number of substrates that are inserted by the bacterial YidC is believed to be much larger than the number of substrates inserted by Oxa1 in mitochondria and Alb3 in chloroplast, partly because YidC can function both independently and cooperatively with the Sec machinery. As an independent insertase, YidC has been shown to insert Foc (subunit c of F_{0}F_{1} ATP synthase), Mscl, the phage proteins M13 procoat and Pf3 coat, and TsSL, a tail-anchored membrane protein (59–65). Interestingly, YidC is able to insert the tail-anchored protein TsSL in bacteria on its own, although a dedicated translocase that is not present in bacteria is employed in eukaryotes (3). Together with the Sec translocase, YidC promotes insertion of the F_{0},F_{1} ATP synthase subunits FoA and FoB, Nuok (NADH-quinone oxidoreductase subunit K), and CyoA (subunit 2 of the cytochrome bo oxidase) (66–71). Interestingly, in a recent study, both SecY and YidC appear to be involved in the assembly of Type IV prepilin in cyanobacterium Synechocystis PCC 6803 (72).

YidC performs its Sec-dependent function as part of a holoenzyme: a super-complex containing YidC, the SecYEG channel, and SecDFYajC (58, 73). In the holoenzyme, YidC is in close proximity to the SecYEG translocation channel (74) where it can interact with membrane protein substrates as they exit the channel through the lateral gate. The SecY lateral gate (TM2b, TM3, TM7, and TM8) region is where membrane protein substrates exit (1) and can be cross-linked to YidC when photoprobe is introduced into the lateral gate region (74). Additional residues in this or other regions may also be important for the YidC and SecY interaction. Recently, Li et al. (75) discovered Gly-355 in TM2 in TM2 and Met-471 in TM4 of the E. coli YidC to be important for YidC-SecY interaction using a synthetic lethal screen. It has been hypothesized that the interaction of YidC with SecYEG is facilitated by SecDFYajC (76). However, cross-linking between the SecY lateral gate and YidC is seen even in the absence of SecDFYajC (74). Moreover, there are populations of the Sec translocon in the membrane that contain only YidC-SecYEG with no SecDFYajC present (77), showing that YidC can interact directly with the SecYEG complex. In the holocomplex, YidC functions to promote the removal of transmembrane segments of inserting membrane proteins from the Sec channel (78), facilitates their integration into the lipid bilayer (79), and acts as an assembly site for multispansing membrane proteins (80). As part of its folding function, YidC plays a direct role in the helix-helix packing of membrane proteins (81, 82). This explains why YidC is required for the folding, but not insertion, of LacY (81, 82) and MalF (83).
and is required for the assembly of the maltose transporter MalFGK (83).

Structure-function studies revealed that the conserved five-TM segment region of the E. coli YidC is critical for its function in membrane protein insertion (84). However, the large periplasmic region of the E. coli YidC is not essential for the insertase function. X-ray crystallographic studies showed that the periplasmic domain has a β-super sandwich fold, which is found in proteins that bind sugars and may play some role in the folding of newly inserted proteins (85, 86). One cryo-electron microscopy study revealed that YidC may function as a channel because YidC was able to form a homodimer (87). The YidC dimer was found to sit at the exit channel of the ribosome near the predicted L23 protein. Another cryo-EM study showed that YidC was bound to the ribosome as a monomer (88). Although it is not entirely clear what the native state is in vivo, data suggest that the functional unit of YidC is a monomer (89).

Recent Insights

In the year 2014, Kumazaki et al. (90) reported the first crystal structure of YidC, a landmark contribution to the membrane biology field. The x-ray structure of YidC was solved from Bacillus halodurans at 2.4 Å resolution. Remarkably, the structure showed that the five membrane-embedded TM segments of YidC contain a hydrophilic cavity within the inner leaflet of the membrane, which is closed from the extracellular side of the membrane (Fig. 1, A and B). Interestingly, the hydrophilic groove is open to the cytoplasm and the lipids of the membrane.

At the entrance of the hydrophilic cavity on the cytoplasmic side of the membrane is a helical hairpin-like domain that may be involved in the initial recruitment of YidC substrates. Intriguingly, the hydrophilic groove contains the strictly conserved positively charged arginine residue Arg-73, which is essential for YidC1 to promote growth in a B. subtilis YidC1 depletion strain and for YidC1 to promote membrane insertion of the single-span MifM protein (90). Arg-73 is proposed to attract the negatively charged residues in the MifM translocated region because substitution of the three negatively charged MifM residues to neutral residues prevented translocation.

In addition to the novel hydrophilic groove, the structure also provided some intriguing information about the dynamics of YidC. Kumazaki et al. (90) isolated two distinct forms of YidC (PDB IDs 3WO6 and 3WO7) that had varied positions of the C1 loop between TMs 1 and 2. The cytoplasmic halves of the TM segments, which make up the groove, also showed positional variance when the two structures are compared. This was corroborated with high crystallographic B-factors in these regions as well as by molecular dynamics simulations. Two other recent studies also pointed to the dynamic and flexible nature of this region (91, 92). Flexibility was observed in membrane vesicles by utilizing intramolecular chemical cross-linking of the E. coli YidC (91). There was cross-linking between the conserved core transmembrane segments with a wide range of different sized cross-linking agents, which suggests that the flexibility occurs in vivo.

FIGURE 1. Crystal structures of YidC homologs. A–D, ribbon representations of the B. halodurans (A and B) and E. coli (C and D) YidC viewed transversely through the membrane with the E. coli P1 domain omitted for simplicity. The conserved arginine residue is shown in purple. The structures are very similar except for the orientation of the C1 domain, shown in cyan.
MINIREVIEW: Membrane Protein Insertases

Additionally, Wickles et al. (92) performed protein evolutionary co-variation analysis, lipid-versus-protein-exposure, and molecular dynamics simulations with the E. coli YidC and were able to determine a model that closely matched the crystallographic structure. The helical hairpin between TM2 and TM3 of the E. coli YidC (TM1 and TM2 of B. halodurans) was also predicted by this study along with the flexibility of this region. Thinning of the membrane was observed during molecular dynamics simulations, which could provide some insight into the translocation mechanism. Given the fact that the hydrophilic cavity spans only the inner leaflet of the membrane, thinning of the membrane could help decrease the energy required to finish translocation across the outer leaflet.

The crystal structure of the Gram-negative E. coli YidC was also reported (PDB ID 3WVF) in late 2014 to 3.2 Å by Kumazaki et al. (93). Like the Gram-positive B. halodurans structure, the five conserved core TMs are tightly packed in the periplasmic half of the membrane and spread out in the cytoplasmic half (Fig. 1, C and D). The E. coli YidC also contains a hydrophilic groove, which is open to the cytoplasm and membrane. The C1 region forms a helical hairpin that is flexible based on crystallographic B-factors, but the arrangement is rotated when compared with the B. halodurans structure. Previously crystallized, the P1 domain was also present, and the large cleft was shown to be oriented away from the membrane, which could allow it to bind substrate proteins or molecules as proposed previously (85, 86).

Interestingly, most of the previously reported substrate contacts (90, 94, 95) are located in the hydrophilic groove between TM3 and TM5 of the E. coli YidC. Others are found on the exterior region of these TMs as well as in the hydrophilic groove located on other TM segments (93) (Fig. 2). It is likely that residues facing the center of the hydrophilic cavity are involved with binding of the hydrophilic translocated region of the substrate, whereas those facing the membrane help insert and laterally integrate the hydrophobic segment of the substrate into the membrane. In addition to reporting the structure, the authors also investigated the importance of the conserved arginine residue using a complementation assay. Hydrophilic groove mutations were able to rescue growth at 37 °C except for T362A, which was previously shown to be inactive (93). However, two cold-sensitive mutants (R366A and R366M) were lethal at 20 °C, which the authors propose illustrates an important role for the conserved positive charge under certain conditions.

The importance of the evolutionarily conserved positive charge for the function of the YidC family was also probed by Chen et al. (96) in a recent publication. The positive charge was determined to be essential for the Gram-positive Streptococcus mutans YidC2 much like the results observed in B. subtilis (90). For E. coli and chloroplast homologs, the charge was not essential for function in an E. coli YidC depletion strain. Mitochondrial Oxa1 had been studied previously, and the conserved charge was determined to be important for activity (97). Variation was observed for the importance of the charge when different substrates were analyzed, which suggests that there are different insertion requirements based on the characteristics of the substrate (96). Additionally, by making deletions and mutations, the C-terminal half of the conserved helical hairpin was found to be important for the activity of E. coli YidC (96).

Insights into how YidC is able to perform multiple roles via interactions with a plethora of Sec substrate proteins have been made recently on various fronts. By isolating a stable holotranslocon (HTL) from E. coli, Schulze et al. (73) were able to determine the components present, the ratios, and some interactions. They observed two versions of the HTL; the first contained one copy of SecYEG, YidC, SecDF, and YajC that functions in membrane protein insertion, whereas the second was a SecYEG dimer that functions in protein export. Interactions were also determined through dithiobis(succinimidyl propionate) chemical cross-linking and were formed between SecD/YidC as well as between SecY, E, and G. The functional state of YidC not in the HTL is still unknown, but it appears as though YidC is a monomer in membranes (89).

The crystal structures of YidC also provide insight into the mechanism by which YidC functions as a translocase (90) (Fig.
FIGURE 3. Model for the insertion of a single-span membrane protein substrate. A, substrate is peripherally associated with the membrane, and YidC is in the resting state. B, the hydrophilic region to be translocated becomes associated with the hydrophilic groove of YidC, possibly via electrostatic interaction. C, a variety of factors is believed to facilitate the release of substrate including: the proton motive force (PMF), hydrophobic interactions between YidC and substrate, and possibly thinning of the membrane by YidC. D, after successful translocation and insertion via a greasy slide, YidC returns to the resting state. This model was adapted from Kumazaki et al. (90).

3). In the initial step, a YidC substrate binds to the membrane (Fig. 3A). Subsequently, the N-tail region of the substrate is recruited to the YidC hydrophilic groove facilitated (Fig. 3, B and C), in some cases, by the interaction of the negatively charged residues in the N-tail with the positively charged arginine in the aqueous cavity. The N-tail is then released from the groove and crosses the outer leaflet of the membrane. This latter step is believed to be catalyzed by the action of the electrical potential (positive side in the periplasmic space) acting on the negative charged residues in the translocated region of the substrate and by hydrophobic interactions between the substrate TM segment and YidC (Fig. 3C). The TM segment of the substrate most likely moves along a greasy slide formed by TM3 and TM5 of the E. coli YidC to form a transmembrane configuration. In the final step, the inserted membrane protein is released from YidC (Fig. 3D). Presumably, a similar mechanism would be used for the YidC substrate TssL; the only difference would be that the C terminus is translocated.

Two recent studies have started to lay the framework for determining the characteristics of substrates that govern the insertion machinery used for integration into the membrane. Zhu et al. (98) proposed that the charge composition of the translocated periplasmic domain or the transmembrane segment could determine which pathway model single-span membrane proteins used for insertion. Hydrophobic TM segments could insert independently, and decreasing the hydrophobicity could lead to a dependence on YidC, SecYEG, or both. A positive charge in a translocated loop required YidC/SecYEG, whereas a negative charge in the loop only required YidC, which corresponds nicely to the mechanism proposed by Kumazaki et al. (90). A more in-depth study was able to predictably alter the requirements of a translocated loop in the M13 procot protein by changing the polarity and charge of the loop (99). Lowering the polarity and number of charges enabled the substrate to translocate via an independent mechanism, whereas increasing the polarity caused YidC and YidC/Sec to be required. By increasing the hydrophobicity of the transmembrane segments, Soman et al. (99) were able to lower the translocate requirement.

These two recent studies build upon the framework laid by earlier studies in the mitochondria as well as E. coli. In the year 2004, Herrmann et al. (100) determined that Oxa1 was important for the insertion of proteins with highly charged domains and, more specifically, for proteins with negative charges in the translocation region. Negative charges in the TM of NuoK were shown to be determinants for YidC, and mutating these residues to lysines caused the protein to become dependent on the Sec complex for insertion (71). The authors also proposed that the conserved role of YidC for respiratory proteins could be due to the fact that respiratory proteins often have negative charges that are essential for function. By utilizing a genome-scale approach, Gray et al. (101) discovered that substrates with charge unbalanced TM segments were significantly more likely to depend on YidC for insertion. However, many of the YidC-dependent proteins identified did not have unbalanced TM segments, and it was proposed that other features also contribute to a protein’s requirement for YidC.

Conclusion

The YidC/Oxa1/Alb3 proteins are a novel group of insertases that function to insert, fold, and assemble proteins into the lipid bilayer. They are particularly important for the assembly of energy-transducing complexes vital for cellular respiration and photosynthesis. Unlike the Sec translocases, the YidC/Oxa1/Alb3 insertases can translocate only short hydrophilic regions of membrane proteins across the membrane and seem to play a more prominent role in the folding of membrane protein substrates.

The emerging structural data suggest that the members of the YidC/Oxa1/Alb3 family of proteins do not function as channels. Rather, they promote the transport of hydrophilic regions of membrane proteins by possessing a hydrophilic groove within the inner leaflet of the membrane. This novel structural feature has not been found to date in other transloca- cases. Recent studies suggest that the hydrophilic cavity most likely recruits the hydrophilic region of the substrate, thus allowing it to transfer halfway across the membrane. It is then released from the groove in a manner not completely understood and crosses the outer leaflet of the membrane. In light of the recent advancements, this is an exciting time for the membrane protein field, and we are poised to finally understand the intricacies of how proteins are inserted into the membrane and how YidC and Sec are able to work cooperatively to accomplish this complex process.

References

1. 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 pro-
MINIREVIEW: Membrane Protein Insertases

1. Denks, K., Vogt, A., Sachelaru, I., Petrimon, N. A., Kudva, R., and Koch, H. G. (2014) The Sec translocon mediated protein transport in prokaryotes and eukaryotes. Mol. Membr. Biol. 31, 58 – 84

2. Hagedoorn, A., Crowley, P. J., Levesque, C. M., Mair, R. W., Cvitkovitch, D. G., Bleiweis, A. S., and Brady, L. J. (2005) Streptococcal viability requires Alb3p. FEBS Lett. 501, 1–5

3. Froehlich, J., Rose, P., and Muller, M. (2012) Twin-arginine-dependent translocation of folded proteins. Philos. Trans. R. Soc. Lond B Biol. Sci. 367, 1029–1046

4. Gerdes, L., Bals, T., Klostermann, E., Karl, M., Philippar, K., Huenken, M., 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

5. Gerdes, L., Bals, T., Klostermann, E., Karl, M., Philippar, K., Huenken, M., Soll, J., and Schuennemann, D. (2006) A second thylakoid membrane-localized Alb3p/Oxa1p/YidC homologue is involved in proper chloroplast biogenesis in Arabidopsis thaliana. J. Biol. Chem. 281, 16632–16642

6. van der Laan, M., Urbanus, M. L., Ten Hagen-Jongman, C. M., Nouwen, N., and Oudega, B., Harms, N., Driessen, A. J., 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

7. Bellaloro, S., Ferris, P., Naver, H., Gohre, V., and Rocheaix, J. D. (2002) Loss of Albino3 leads to the specific depletion of the light-harvesting system. Plant Cell 14, 2303–2314

8. Ossenbuhl, F., Gohre, V., Meurer, J., Krieger-Liszkay, A., Rocheaix, J. D., and Eichacker, L. A. (2004) Efficient assembly of photosystem II in Chlamydomonas reinhardtii requires Alb3p.1p, a homolog of Arabidopsis ALBINO3. Plant Cell 16, 1790–1800

9. Spence, E., Bailey, S., Nenninger, A., Muller, S. G., and Robinson, C. (2004) A homolog of Albino3/Oxa is essential for thylakoid biogenesis in the cyanobacterium Synechocystis sp. PCC6803. J. Biol. Chem. 279, 55792–55800

10. Dong, Y., Palmer, S. R., Hasona, A., Nagamori, S., Kaback, H. R., Dalbey, R. E., and Brady, L. J. (2008) Functional overlap but lack of complete cross-complementation of Streptococcus mat and Escherichia coli YidC orthologs. J. Bacteriol. 190, 2458–2469

11. Benz, M., Bals, T., Gügel, I. L., Piotrowski, M., Kuhn, A., Schüennemann, D., Soll, J., and Ankele, E. (2009) Alb4 of Arabidopsis promotes assembly and stabilization of a non chlorophyll-binding photosynthetic complex, the CF, CF., ATP synthase. Mol. Plant 2, 1410–1424

12. Jiang, F., Li, Y., Moore, M., Chen, M., Rohl, T., Van Wijk, K. J., De Gier, J. W., Henry, R., and Dalbey, R. E. (2002) Chloroplast YidC homolog Albino3 can functionally complement the bacterial YidC depletion strain and promote membrane insertion of both bacterial and chloroplast thylakoid proteins. J. Biol. Chem. 277, 19281–19288

13. van Bloois, E., Nagamori, S., Koningstein, G., Ullers, R. S., Preuss, M., Oudega, B., Harms, N., Kaback, H. R., Herrmann, J. M., Luirink, J. (2005) The Sec-independent function of Escherichia coli YidC is evolutionary-conserved and essential. J. Biol. Chem. 280, 12996–13003

14. He, S., and Fox, T. D. (1997) Membrane translocation of mitochondrially coded Cox2p: distinct requirements for export of N and C termini and dependence on the conserved protein Oxa1p. Mol. Biol. Cell 8, 1449–1460

15. Hell, K., Herrmann, J., Pratte, E., Neupert, W., and Stuart, R. A. (1997) Oxa1p mediates the export of the N- and C-termini of pCoXII from the mitochondrial matrix to the intermembrane space. FEBS Lett. 418, 367–370

16. Hell, K., Neupert, W., and Stuart, R. A. (2001) Oxa1p acts as a general membrane insertion machinery for proteins encoded by mitochondrial DNA. EMBO J. 20, 1281–1288

17. Hell, K., Herrmann, J. M., Pratte, E., Neupert, W., and Stuart, R. A. (1998) Oxa1p, an essential component of the N-tail protein export machinery in mitochondria. Proc. Natl. Acad. Sci. U.S.A. 95, 2250–2255

18. Bohnert, M., Rehling, P., Gutiérrez, B., Herrmann, J. M., Pfänder, N., and van der Laan, M. (2010) Cooperation of stop-transfer and conservative sorting mechanisms in mitochondrial protein transport. Curr. Biol. 20, 1227–1232

19. Jia, L., Dienhart, M., Schramp, M., McCauley, M., Hell, K., and Stuart, R. A. (2003) Yeast Oxa1 interacts with mitochondrial ribosomes: the importance of the C-terminal region of Oxa1. EMBO J. 22, 6438–6447
MINIREVIEW: Membrane Protein Insertases

40. Jia, L., Kaur, J., and Stuart, R. A. (2009) Mapping of the Saccharomyces cerevisiae Oxa1 mitochondrial ribosome interface and identification of Mrpl40, a ribosomal protein in close proximity to Oxa1 and critical for oxidative phosphorylation complex assembly. *Eukaryot. Cell* 8, 1792–1802

41. Kaur, J., and Stuart, R. A. (2011) Truncation of the Mrp20 protein reveals new ribosome-assembly subcomplex in mitochondria. *EMBO Rep.* 12, 950–955

42. Keil, M., Bareth, B., Woelhalt, M. W., Peleh, V., Prestele, M., Rehling, P., and Herrmann, J. M. (2012) Oxa1-ribosome complexes coordinate the assembly of cytochrome c oxidase in mitochondria. *J. Biol. Chem.* 287, 34484–34493

43. Saracco, S. A., and Fox, T. D. (2002) Cox18p is required for export of the mitochondrially encoded Saccharomyces cerevisiae Cox2p C-tail and interacts with Pnt1p and Mss2p in the inner membrane. *Mol. Biol. Cell* 13, 1122–1131

44. Fimura, H. L., Broadley, S. A., and Fox, T. D. (2007) Translocation of mitochondrially synthesized Cox2 domains from the matrix to the intermembrane space. *Mol. Cell Biol.* 27, 4664–4673

45. Koppen, M., and Langer, T. (2007) Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases. *Crit. Rev. Biochem. Mol. Biol.* 42, 221–242

46. Fimura, H. L., Dunham, M. J., Saracco, S. A., Butler, C. A., Kelly, J. A., and Fox, T. D. (2009) Translocation and assembly of mitochondrially encoded Saccharomyces cerevisiae cytochrome c oxidase subunit Cox2p by Oxa1 and Yme1 in the absence of Cox18. *Genetics* 182, 519–528

47. Moore, M., Harrison, M. S., Peterson, E. C., and Henry, R. (2000) Chloroplast Oxa1p homolog abino3 is required for post-translational integration of the light harvesting chlorophyll-binding protein into thylakoid membranes. *J. Biol. Chem.* 275, 1529–1532

48. Schuenemann, D., Gupta, S., Persello-Cartieaux, F., Klimyuk, V. I., Jones, J. D. G., Nussaume, L., and Hoffman, N. E. (1998) A novel signal recognition particle RNA is conserved in plastids of a wide range of photosynthetic organisms. *Science* 281, 893–901

49. Moore, M., Harrison, M. S., Peterson, E. C., and Henry, R. (2000) Chloroplast Oxa1p homolog abino3 is required for post-translational integration of the light harvesting chlorophyll-binding protein into thylakoid membranes. *J. Biol. Chem.* 275, 1529–1532

50. Keil, M., Bareth, B., Woellhaf, M. W., Peleh, V., Prestele, M., Rehling, P., and Herrmann, J. M. (2012) Oxa1-ribosome complexes coordinate the assembly of cytochrome c oxidase in mitochondria. *J. Biol. Chem.* 287, 34484–34493

51. Kosterev, E., Droste Gen Helling, I., Carde, J. P., and Schuennemann, D. (2002) The thylakoid membrane protein ALB3 associates with the cpSecY-translocon in Arabidopsis thaliana. *Biochem. J.* 368, 777–781

52. Pasch, J. C., Nickelsen, J., and Schuennemann, D. (2005) The yeast split-ubiquitin system to study chloroplast membrane protein interactions. *Appl. Microbiol. Biotechnol.* 69, 440–447

53. Göhr, V., Ossenbühl, F., Crévieux, M., Eichacker, L. A., and Rocheaix, J. D. (2006) One of two α3 proteins is essential for the assembly of the photosystems and for cell survival in Chlamydomonas. *Plant Cell* 18, 1454–1466

54. Scotti, P. A., Urbanus, M. L., Brunner, J., de Gier, J. W., and von Heijne, G., van der Does, C., Driessen, A. J., Oudega, B., Luijirk, J. (2000) YidC, the Escherichia coli homologue of mitochondrial Oxa1p, is a component of the Sec translocon. *EMBO J.* 19, 542–549

55. Samuels, J. C., Chen, M., Jiang, F., Möller, I., Wiedmann, M., Kuhn, A., Phillips, G. J., and Dalbey, R. E. (2000) YidC mediates membrane protein insertion in bacteria. *Nature* 406, 637–641

56. Pasch, J. C., Nickelsen, J., and Schuennemann, D. (2005) The yeast split-ubiquitin system to study chloroplast membrane protein interactions. *Appl. Microbiol. Biotechnol.* 69, 440–447

57. GÖhr, V., Ossenbühl, F., Crévieux, M., Eichacker, L. A., and Rocheaix, J. D. (2006) One of two α3 proteins is essential for the assembly of the photosystems and for cell survival in Chlamydomonas. *Plant Cell* 18, 1454–1466

58. Scotti, P. A., Urbanus, M. L., Brunner, J., de Gier, J. W., and von Heijne, G., van der Does, C., Driessen, A. J., Oudega, B., Luijirk, J. (2000) YidC,
MINIREVIEW: Membrane Protein Insertases

meric complex with YidC. Mol. Microbiol. 44, 1397–1405
77. Götzke, H., Palombo, L., Muhheim, C., Perrody, E., Genevaux, P., Kudva, R., Müller, M., and Dalbey, R. (2012) Both YidC and SecY are required for translocation of the periplasmic loops 1 and 2 of the multispanning membrane protein TatC. J. Mol. Biol. 424, 354–367
78. Urbanus, M. L., Scotti, P. A., Fröderberg, L., Sääf, A., de Gier, J. W. L., Brunner, J., Samuelson, J. C., Dalbey, R. E., Oudega, B., and Lurink, J. (2001) Sec-dependent membrane protein insertion: sequential interaction of nascent FtsQ with SecY and YidC. EMBO Rep. 2, 524–529
80. Beck, K., Eisner, G., Trescher, D., Dalbey, R. E., Brunner, J., and Müller, M. (2001) YidC, an assembly site for polytopic Escherichia coli membrane proteins located in immediate proximity to the SecYE translocon and lipids. EMBO Rep. 2, 709–714
82. Zhu, L., Kaback, H. R. (2004) Role of YidC in folding of polytopic membrane proteins. J. Cell Biol. 165, 53–62
83. Wagner, S., Pop, O. I., Haan, G. J., Baars, L., Koningstein, G., Klepsch, M. M., Genevaux, P., Lurink, J., and de Gier, J. W. (2008) Biogenesis of MalF and the MalFGK2 maltose transport complex in Rhodopirellula baltica C-terminal region of YidC from MalF and the MalFGK2 maltose transport complex in Rhodopirellula baltica C-terminal region of YidC from J. Biol. Chem. 283, 28180–28194
84. Wagner, S., Pop, O. I., Haan, G. J., Baars, L., Koningstein, G., Klepsch, M. M., Genevaux, P., Lurink, J., and de Gier, J. W. (2008) Biogenesis of MalF and the MalFGK2 maltose transport complex in Escherichia coli requires YidC. J. Biol. Chem. 283, 17881–17890
85. Oliver, D. C., and Paetzel, M. (2008) Crystal structure of the major periplasmic domain of the bacterial membrane protein assembly facilitator YidC. J. Biol. Chem. 283, 5208–5216
86. Ravaud, S., Stjepanovic, G., Wild, K., and Sinning, I. (2008) The crystal structure of the periplasmic domain of the Escherichia coli membrane protein insertase YidC contains a substrate binding cleft. J. Biol. Chem. 283, 48965–48972
87. Kohler, R., Boehringer, D., Greber, B., Bingel-Erlenmeyer, R., Collinson, I., Schaffitzel, C., and Ban, N. (2009) YidC and Oxa1 form dimeric insertion pores on the translating ribosome. Mol. Cell 34, 344–353
88. Setl, I., Wickles, S., Beckmann, R., Kuhn, A., and Kiefer, D. (2014) The C-terminal regions of YidC from Rhodopirellula baltica and Oceanicaislus alexandrii bind to ribosomes and partially substitute for SRP receptor function in Escherichia coli. Mol. Microbiol. 91, 408–421
89. Kedrov, A., Sustarsic, M., de Keyser, J., Cauumanns, J. J., Wu, Z. C., and Driessen, A. J. (2013) Elucidating the native architecture of the YidC: ribosome complex. J. Mol. Biol. 425, 4112–4124
90. Kumazaki, K., Chiba, S., Takemoto, M., Furukawa, A., Nishiyama, K.-I., Sugano, Y., Mori, T., Dohmae, N., Hirata, K., Nakada-Nakura, Y., Matuurana, A. D., Tanaka, Y., Mori, H., Sugita, Y., Azisaka, F., Ito, K., Ishitani, R., Tsukazaki, T., and Nureki, O. (2014) Structural basis for Sec-independent membrane protein insertion by YidC. Nature 509, 516–520
91. Hennon, S. W., and Dalbey, R. E. (2014) Cross-linking-based flexibility and proximity relationships between the TM segments of the Escherichia coli YidC. Biochemistry 53, 3278–3286
92. Wickles, S., Singharyo, A., Andreani, J., Seemayer, S., Bischoff, L., Berninghausen, O., Soeding, J., Schulten, K., van der Sluis, E. O., and Beckmann, R. (2014) Structural model of the active ribosome-bound membrane protein insertase YidC. Elife 3, e03035
93. Kumazaki, K., Kishimoto, T., Furukawa, A., Mori, H., Tanaka, Y., Dohmae, N., Ishitani, R., Tsukazaki, T., and Nureki, O. (2014) Crystal structure of Escherichia coli YidC, a membrane protein chaperone and insertase. Sci. Rep. 4, 7299
94. Yu, Z., Koningstein, G., Pop, A., and Lurink, J. (2008) The conserved third transmembrane segment of YidC contacts nascent Escherichia coli inner membrane proteins. J. Biol. Chem. 283, 34635–34642
95. Klenner, C., and Kuhn, A. (2012) Dynamic disulfide scanning of the membrane-inserting PEB coat protein reveals multiple YidC substrate contacts. J. Biol. Chem. 287, 3769–3776
96. Chen, Y., Soman, R., Shannumag, S. K., Kuhn, A., and Dalbey, R. E. (2014) The role of the strictly conserved positively charged residue differs among the Gram-positive, Gram-negative and chloroplast YidC homologs. J. Biol. Chem. 289, 35656–35667
97. Lemaire, C., Guibert-Grandmougin, F., Angles, D., Dujardin, G., and Bonnefoy, N. (2004) A yeast mitochondrial membrane methyltransferase-like protein can compensate for oxa1 mutations. J. Biol. Chem. 279, 47464–47472
98. Zhu, L., Wasey, A., White, S. H., and Dalbey, R. E. (2013) Charge-composition features of model single-span membrane proteins that determine selection of YidC and SecYEG translocase pathways in Escherichia coli. J. Biol. Chem. 288, 7704–7716
99. Soman, R., Yuan, J., Kuhn, A., and Dalbey, R. E. (2014) Polarity and charge of the periplasmic loop determine the YidC and sec translocase requirement for the M13 procoat lep protein. J. Biol. Chem. 289, 1023–1032
100. Herrmann, J. M., and Bonnefoy, N. (2004) Protein export across the inner membrane of mitochondria: the nature of translocated domains determines the dependence on the Oxa1 translocase. J. Biol. Chem. 279, 2507–2512
101. Gray, A. N., Henderson-Frost, J. M., Boyd, D., Sharafi, S., Niki, H., and Goldberg, M. B. (2011) Unbalanced charge distribution as a determinant for dependence of a subset of Escherichia coli membrane proteins on the membrane insertase YidC. MBio 2, e00238–11