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Review

Inserting membrane proteins: The YidC/Oxa1/Alb3 machinery in bacteria, mitochondria, and chloroplasts☆

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ABSTRACT

The evolutionarily conserved YidC/Oxa1p/Alb3 family of proteins plays important roles in the membrane biogenesis in bacteria, mitochondria, and chloroplasts. The members in this family function as novel membrane protein insertases, chaperones, and assembly factors for transmembrane proteins, including energy transduction complexes localized in the bacterial and mitochondrial inner membrane, and in the chloroplast thylakoid membrane. In this review, we will present recent progress with this class of proteins in membrane protein biogenesis and discuss the structure/function relationships. This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

Contents

1. Introduction

In all living cells, proteins, which are synthesized in the cytoplasm of the cell, must be transported to their correct location in order to function properly. In eukaryotic cells, proteins are sorted from the cytoplasm to membrane-enclosed organelles such as the nucleus, endoplasmic reticulum, peroxisome, mitochondria, and chloroplasts. In prokaryotes, proteins are transported from the cytoplasm to the cell surface. During the last decade, major progress has been made in understanding how these transport processes are accomplished.

In this review, we will focus on the universally conserved YidC/Oxa1/Alb3 pathways which play important roles in the insertion of proteins into the inner membrane of bacteria and mitochondria and the thylakoid membrane in chloroplasts. Before we describe the YidC/Oxa1/Alb3 pathways, we will review the different pathways for localization of proteins in mitochondria, chloroplasts, and bacteria. For a comprehensive review of protein import into mitochondria and chloroplasts and protein export in bacteria, see References [10,15,42].

2. Protein import and export systems

In eukaryotic cells, many mitochondrial proteins are synthesized in the cytoplasm as higher molecular weight precursor proteins and imported into mitochondria (Fig. 1; top left panel). The translocase of

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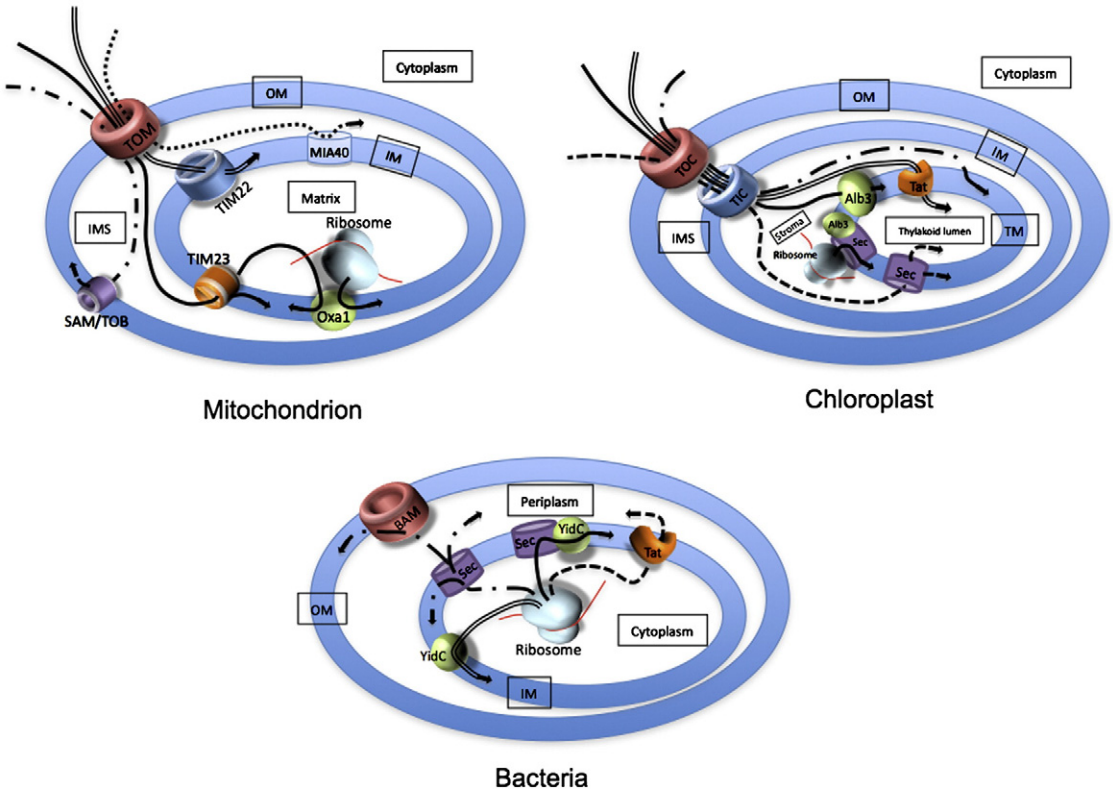

Fig. 1. Summary of the protein import and export systems. Import of proteins to various compartments in mitochondria (top left), chloroplasts (top right), and protein export in bacteria (bottom). Protein traffic is illustrated by different lines, as described in the paper. OM: outer membrane; IM: inner membrane; IMS: intramembrane space; TM: thylakoid membrane.

the outer membrane (TOM complex) facilitates the translocation of proteins across the mitochondrial outer membrane into the intermembrane space. At this stage, proteins can continue their journey to the mitochondrial matrix, to the inner membrane or be reinserted into the outer membrane. For example, the β-barrel proteins in the outer membrane are transported from the mitochondrial intermembrane space (IMS) and reinserted into the outer membrane by the sorting and assembly machinery of the outer membrane (SAM/TOB complex) [50,68]. On the other hand, some proteins remain in the intermembrane space where, in some cases, they interact with the mitochondrial intermembrane space assembly (MIA40) machinery to form disulfide bonds. Transport from the intermembrane space to the matrix is facilitated by the TIM 23 complex.

Protein sorting to the mitochondrial inner membrane can occur by three pathways. The metabolite carrier family exploits the translocase of the inner membrane (TIM22 complex) to insert into the mitochondrial inner membrane. These proteins have internal targeting signals and interact with the small TIM chaperones in the intermembrane space before insertion. The insertion of the carrier proteins into the mitochondrial inner membrane requires the membrane electrical potential. Alternatively, inner membrane proteins use the TIM23 complex to insert and then laterally integrate into the inner membrane. These proteins contain a lateral sorting signal and are typically single transmembrane proteins [25]. Finally, proteins can use a bacterial-like Oxa1 pathway for insertion into the inner membrane from the matrix compartment. Either these proteins are imported into the mitochondrial matrix by the TIM23 complex and then reinserted into the inner membrane or they are cotranslationally inserted as they are being synthesized by mitochondrial ribosomes; for review, see References [7,67].

The majority of chloroplast proteins, like mitochondrial proteins, are encoded by the nuclear genome and synthesized in the cytoplasm as higher molecular weight precursors with a chloroplast transit peptide. Chloroplast proteins are imported into six possible suborganellar destinations: outer membrane, inner membrane, thylakoid membrane, intramembrane space, stroma, and thylakoid lumen (Fig. 1; top right panel). Crossing the chloroplast outer and inner membrane is catalyzed by the translocase of the outer envelope membrane of chloroplast (TOC) and the translocase of the inner envelope membrane of chloroplast (TIC). After removal of the transit peptide by stroma processing peptidase, stroma proteins remain in this compartment.

The Sec and Tat protein transport pathways are used to export proteins to the chloroplast thylakoid lumen [12]. Many of these proteins are synthesized in precursor forms with signal sequences having a bipartite structure comprised of a stroma-targeting signal and a thylakoid lumen signal resembling a bacterial signal peptide. The amino-terminal signal directs the protein to the chloroplast stroma compartment where the stroma transit peptide is removed. The second signal peptide targets the protein either to the Sec machinery or the Tat machinery depending upon the nature of the signal peptide. Although the Sec and Tat signals are similar in structure, the Tat signal peptides typically contain an RR motif preceding the hydrophobic domain. The Sec pathway exports proteins across the thylakoid membrane in an unfolded state, whereas the Tat pathway is very unusual and can export proteins in a folded state to the thylakoid lumen. The Sec translocase can also insert proteins into the thylakoid membrane [59], although the majority of tested proteins insert by the unassisted pathway [110]. The extent to which these pathways are also used for stroma-synthesized proteins encoded by the chloroplasts genome is not clear. Finally, the light-harvesting chlorophyll a,b binding proteins (LHCPs) are targeted to the membrane posttranslationally by the chloroplast SRP and are inserted by the Alb3 pathway [34].

Like chloroplast, the bacterial Sec and Tat pathways are responsible for exporting proteins across the inner membrane to the periplasmic space (Fig. 1; bottom panel) [118]. The Sec pathway is

the main pathway employed for protein export to the periplasmic space, while the Tat pathway in *Escherichia coli* exports a limited number of folded proteins which typically contain metal cofactors. The Sec translocase is also utilized in bacteria to insert the majority of proteins into the inner membrane. Many of the integral membrane proteins are targeted to the inner membrane by the SRP pathway [96]. Sec-independent proteins are inserted into the inner membrane by the YidC pathway; for review, see References [43,49,112].

After translocation across the bacterial inner membrane, β-barrel proteins are directed and inserted into the outer membrane by the β-barrel assembly machinery (BAM) similar to the SAM (TOB) complex used to insert outer membrane proteins from the intramembrane space of mitochondria [46,107]. The bacterial BamA (YaeT, Omp85) protein is homologous to the mitochondrial Sam50 (Tob55) [22,68]. The other components of the BAM complex are not homologous. In bacteria, the complex contains four periplasmically exposed membrane-associated lipoproteins (BamB–BamE) (YfgL, NlpB, YfiO, SmpA, respectively) [85,111], whereas in mitochondria, the complex contains the non-lipoproteins Sam37 and Sam35 that are associated with the SAM complex on the cytosolic face of the outer membrane [50,56,106]. Transport of bacterial outer membrane lipoproteins from the inner membrane to the outer membrane requires the Lol system, a bacterial-specific pathway [93]. It involves the LolABCDE protein components.

The conserved pathways found in bacteria, mitochondria and chloroplasts are due to the fact that mitochondria and chloroplasts are derived from bacteria by an endosymbiotic event [82]. Mitochondria are believed to have evolved from an ancient purple bacteria and chloroplasts from an ancient cyanobacterium which was engulfed by an eukaryotic ancestor. However, the mitochondrial and chloroplasts genomes have been greatly reduced in size during evolution and the genes transferred to the host nucleus. This leads to the need for protein import into the organelle and the need for the TIM/TOM and the TIC/TOC complexes. The majority of mitochondria lost the Sec, SRP and Tat components after the endosymbiotic event [24], whereas chloroplasts retained these components. Only the mitochondria of certain eukaryotes such as *Reclinomonas americana* contain Sec homologs [51].

3. Discovery of the mitochondrial Oxa1 and its homologs in chloroplasts and bacteria

In 1994, an Oxa1 mutant that showed defects in cytochrome *c* oxidase assembly was discovered [2,6]. In 1997, Oxa1 was shown to be essential for the translocation of the N-terminal tail of subunit II of cytochrome *c* oxidase (Cox II) [30,32]. The combined data indicated that Oxa1 is important for the formation of the cytochrome *c* oxidase complex and that Oxa1 functions as a translocase.

Homologs of Oxa1 were soon found in bacteria and chloroplasts. The mitochondrial (Oxa1) and chloroplasts (Alb3) homologs were predicted to span the membrane five times. The gram-negative bacterial (YidC) homolog has an extra transmembrane segment and spans the membrane six times (Fig. 2) [36,75,90]. Each of the homologs in bacteria, mitochondria, and chloroplasts has five C-terminal conserved transmembrane segments. YidC in gram-negative bacteria typically has a large periplasmic domain between transmembrane segments (TMs) 1 and 2, whereas YidC in gram-positive bacteria lack such a large domain and span the membrane only five times [69,114]. Because of the existence of these homologs in bacteria and chloroplasts, scientists soon tested whether membrane insertion into the chloroplast thylakoid membrane and inner membrane in bacteria require this family of proteins. Indeed, the bacterial YidC and chloroplast Alb3 were shown to play a role in membrane protein insertion in bacteria and chloroplast, respectively [57,77].

4. The bacterial YidC insertase

In 2000, there were two landmark studies that showed that YidC functions in membrane protein insertion in bacteria. Scotti et al. [83] reported that YidC copurified with the Sec translocase and that YidC could be photo-cross-linked to nascent membrane proteins. Concurrently, Samuelson et al. [77] demonstrated that YidC is critical for the insertion of the Sec-independent M13 procoat protein and facilitates the insertion of Sec-dependent proteins, although to a lesser degree. The combined data showed YidC is a new translocase component that inserts transmembrane segments of newly synthesized proteins into the membrane [89].

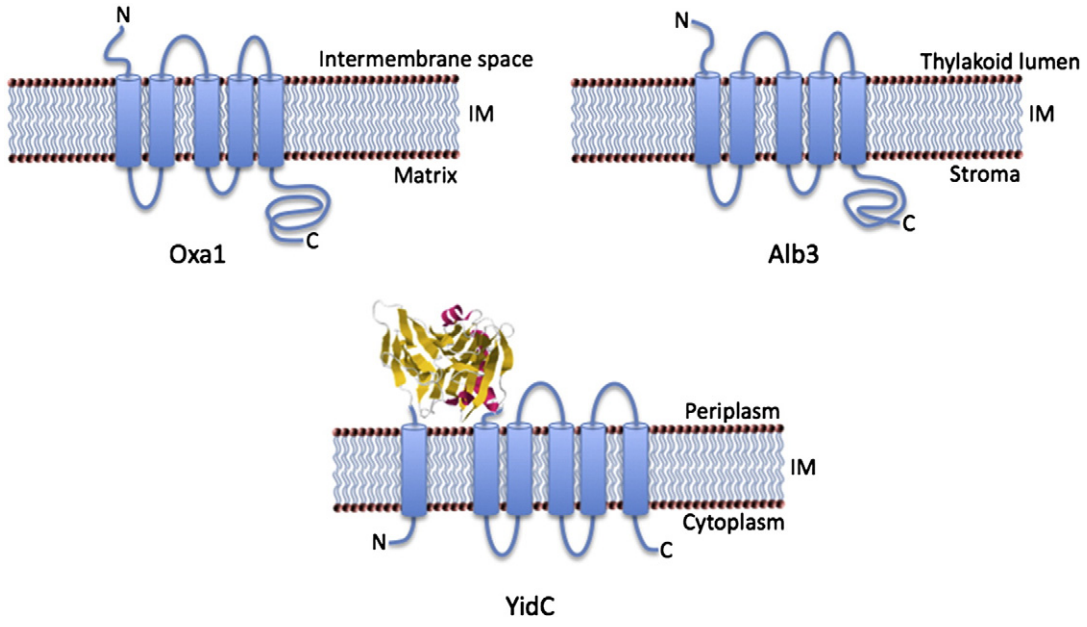

Fig. 2. Topology of YidC/Oxa1/Alb3 family of proteins. Topology was mapped using alkaline phosphatase/β-galactosidase fusions for YidC [75], protease mapping for Oxa1 [80], or predicted for Alb3 protein. For YidC, the structure of the periplasmic domain (PDB 3BLC) has been determined [65,74]. In the structural domain, yellow represent the β-sheets and pink corresponds to α-helices.

The key machinery for inserting proteins into the inner membrane in *E. coli* is the Sec apparatus [104]. The bacterial Sec translocase is comprised of the trimeric SecYEG and SecDFYajC membrane complexes, the peripheral membrane protein SecA, and YidC [15]. The accessory component YidC binds to the Sec translocase by the interaction of YidC with SecDFYajC complex which acts as a bridge by bringing it to the SecYEG complex [63]. YidC has been shown to be required for membrane insertion of several Sec substrates, including ATP synthase subunit a (Foa), cytochrome bo_3 oxidase CyoA subunit and NADH dehydrogenase I subunit K [9,16,73,101,115,116].

In addition to acting as a membrane integrase for Sec-dependent substrates, YidC can also act as a chaperone and as an assembly site for membrane protein folding (Fig. 3; left panel). Photo-cross-linking studies revealed that the transmembrane segment of FtsQ interacts first with the SecYEG channel and then with YidC before integrating into the lipid environment [97]. YidC was proposed to stabilize TMs after they leave the SecYEG translocation channel and mediate their partitioning into the lipid environment. In addition, YidC was shown to contact at least three TM segments of mannitol permease during the membrane insertion [3]. This interaction of YidC with TM segments during membrane insertion of polytopic membrane proteins may be the reason why YidC depletion affects the folding of the Lac permease and MalF protein [60,105]. Alternatively, YidC may be required for insertion of one or more of the periplasmic loops of these multispanning membrane proteins.

YidC can also act as an insertase, independent of the Sec translocase [84,103] (Fig. 3; right panel). It has been shown the Sec-independent M13 and Pf3 phage coat proteins [11,77], subunit c of the ATP synthase [99,103,115,116], and possibly MscL [17,70] require YidC for membrane protein insertion. When YidC is depleted using an *araBAD* promoter controlled YidC strain, the Sec-independent proteins are strongly inhibited in membrane insertion [77,78]. Second, YidC proteoliposomes are sufficient to insert the Pf3 coat and subunit c protein [84,103]. Third, significant levels of YidC are believed to be free of the SecYEG complex since YidC is much more abundant than the Sec components [98]. While the YidC substrates procoat and Pf3 coat do not require SRP for insertion, MscL protein does [17].

What is the driving force for the insertion of proteins by the YidC pathway? We propose that membrane protein insertion is driven by the binding of the substrate to the membrane-bound YidC chaperone. We hypothesize that YidC binding to the substrate transiently stabilizes the membrane inserted form, thereby inducing the hydrophobic region of the membrane protein substrate to assume an α-helical transmembrane configuration. The dissociation of the substrate from YidC would promote the partitioning of the substrate into the lipid environment and the translocation of the hydrophilic region across the membrane. Without YidC, the energy activation barrier is too high for insertion of the membrane protein into the lipid bilayer and would occur too slowly. The driving force for membrane insertion by YidC chaperones is different from most translocation systems. In bacteria, ATP hydrolysis is the energy source driving posttranslational translocation of SecA-dependent proteins while GTP hydrolysis (in protein synthesis) drives cotranslational insertion into the SecYEG protein-conducting channel as they are being synthesized on the ribosome. To the best of our knowledge, YidC family members are unique in that their insertase/chaperone activity is located in the portion of the protein that is completely embedded within the membrane and performs this function without nucleotide triphosphate hydrolysis.

In bacteria, YidC family members play a critical role in the assembly of respiratory complexes. Van der Laan et al. [102] showed that when YidC is depleted in *E. coli*, there is a reduction in the activity of the cytochrome bo_3 oxidase and F1Fo ATP synthase. This is, in part, because membrane insertion of subunit II of cytochrome bo_3 oxidase and subunits a and c of the F1Fo ATP synthase is inhibited [49]. As expected with a defect in the assembly of the energy transducing complexes, there is a reduction in the proton motive force (PMF). Concomitantly, there is a strong induction of the phage shock protein response as YidC is depleted in bacteria [41,102]. However, the induction of PspA is dramatically higher when YidC is depleted [108] compared to when PMF is collapsed by the addition of a protonophore [109].

Most gram-positive bacteria, in contrast to gram-negative bacteria, have two YidC homologs. In *Bacillus subtilis*, the YidC homologs are called SpoIIIJ and YdjG [92]. Deletion of either one does not result in cell death, although deletion of both is lethal [92]. *Streptococcus mutans* contains YidC1 and YidC2 [29]. Neither one is essential for viability, although YidC2 is required for growth under acid stress conditions. Interestingly, this YidC2 requirement under acid stress condition is similar to the phenotype observed with the *S. mutans ffh* or *ftsY* knockout strain [29]. YidC2 is required in *S. mutans* for the F1Fo ATP synthase activity [29] which is most likely needed under acid stress condition since the ATP synthase is believed to use its ATP activity to pump protons across the membrane [4]. Remarkably, the *E. coli* YidC can substitute for YidC2 in *S. mutans* and support growth even under acid stress conditions [14]. In addition, either *S. mutans* YidC1 or YidC2 can substitute for YidC in *E. coli* and support growth in liquid medium as well as promote membrane insertion of YidC-dependent substrates.

The YidC/Oxa1/Alb3 family of proteins, despite operating in different membrane systems, has similar functions, although there are some species-specific differences. The chloroplasts Alb3 and mitochondrial Oxa1 can replace YidC in *E. coli* and function in membrane protein

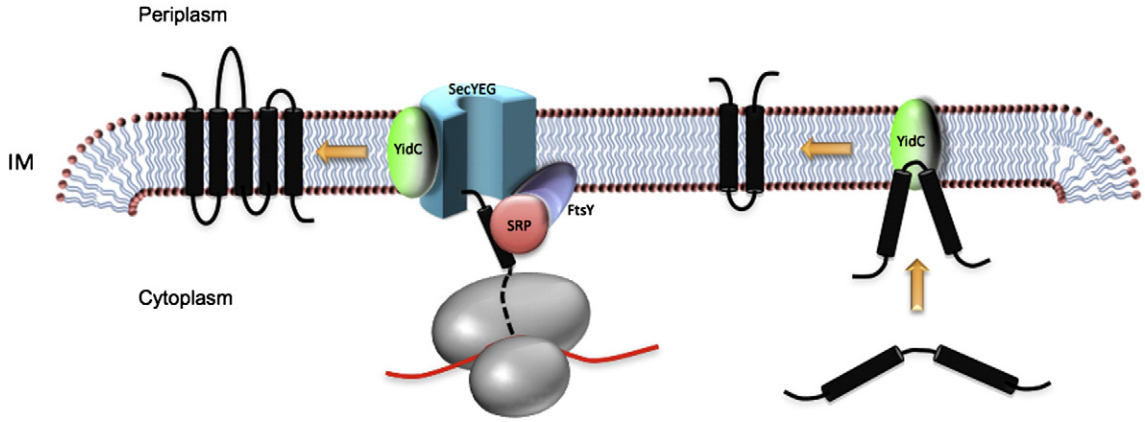

Fig. 3. Function of YidC in the bacterial system. YidC operates as an independent insertase (right) or cooperates with the SecYEG as an insertase and foldase (left). Substrates of the YidC-only pathway include Pf3 coat, M13 procoat, subunit c of ATP synthase, and MscL. Substrates of SecYEG/YidC pathway include subunit a of ATP synthase, CyoA, Lac permease, and MalF. In the Sec pathway, YidC is believed to stabilize a TM segment after it is released from SecYEG channel and mediate its partitioning into the lipid bilayer.

insertion [39,100]. The *E. coli* YidC was interchangeable with the mitochondrial Oxa1 and supports insertion of Oxa1 substrates into the mitochondrial inner membrane as long as the C-terminal matrix domain is appended to YidC [71]. Previously, the C-terminal matrix domain of Oxa1 was shown to constitute the ribosomal binding domain of Oxa1 [37,91]. Interestingly, the *S. mutans* YidC2 can function in place of Oxa1 even without appending the C-terminal domain [20]. The *S. mutans* YidC2 contains a large cytoplasmic domain that can also bind to ribosome [20].

The structural functional relationships of the *E. coli* YidC have been probed by mutagenesis studies to define the regions of YidC that are critical for its function [40]. The majority of the periplasmic domain of YidC could be deleted without any impact on the function of YidC. However, the function of YidC is abolished with deletions in the conserved C-terminal region containing the five TM segments. Remarkably, TM4 and TM5 of the *E. coli* YidC could be replaced with TM1 and TM2 of leader peptidase, respectively. Also, mutagenesis studies showed that TM2, TM3, and TM6 are important for YidC function; mutations in these membrane-spanning regions impacted the ability of YidC to support growth and membrane protein insertion of substrates [40]. In addition, cold-sensitive mutations were isolated in TM3 of YidC [119]. The c.s. mutants were used to isolate suppressor mutations that restored growth at the cold-sensitive temperature. A suppressor mutation was isolated in TM2 of YidC, suggesting that there is an interaction between TM2 and TM3 [119].

The importance of TM3 of YidC may have to do with this region forming part of the substrate binding region [44,117]. Klenner et al. [44] found that TM1 and TM3 of YidC come into close contact to the transmembrane segment of the Pf3 coat protein using an *in vivo* disulfide cross-linking approach. TM3 of YidC was also found to interact with the transmembrane segment of several other substrates [117]. In this experiment, nascent membrane proteins were trapped in the process of membrane insertion using an *in vitro* truncated mRNA approach. Efficient cross-linking could be achieved between cysteines located within YidC TM3 (at P425, L426, Q429) and a cysteine located at various positions of the transmembrane region (at residues 36, 39, 40, 41, and 42) of the FtsQ substrate. Yu et al. [117] hypothesized that one side of the TM3 α-helix of YidC is a substrate-docking region that interacts with the substrate, and the transmembrane segment of the nascent chain is flexible in its interaction with YidC.

A clue to how YidC may function at the molecular level in membrane protein insertion has come from cryoelectron microscopy analysis and cross-linking experiments. Based on these experiments, it was proposed that a dimer of YidC forms an insertion pore on translating ribosomes [48]. Substrate is predicted to insert at the region formed at the interface of two YidC monomers in a head-to-tail fashion with TM2 and TM3 of one subunit located nearby TM2 and TM3 of the neighboring subunit. TM3 of one YidC monomer can be cross-linked to TM3 of the other monomer. Likewise, TM1 (corresponding to the YidC TM2) of one Oxa1 monomer can be cross-linked to TM1 of the other Oxa1 molecule. Such a dimeric pore with TM2 and TM3 playing important roles possibly explains why mutations in TM2 and TM3 impair YidC activity [40]. This model is also supported by recent studies showing that TM3 is in close proximity to the substrate during membrane insertion as mentioned in the previous paragraph [44,117] and the fact that TM2 and TM3 play important roles in YidC function [40,119]. Moreover, some purified YidC appears as a dimer on a blue native polyacrylamide gel [63].

Recently, the periplasmic region of YidC was solved to high resolution by X-ray crystallography [65,74]. Interestingly, the periplasmic domain contains a β super sandwich fold (Fig. 2). The structure has similarity to proteins that are involved in sugar binding [65]. The structure of the periplasmic domain provides clues to how YidC interacts with SecDFyajC since it was discovered that YidC residues 215–265 of the periplasmic region is sufficient for binding to SecF [113]. This structure reveals that residues 215–265 correspond to the edge of the β sandwich structure possessing a surface enriched in acidic residues adjacent to a surface enriched in basic residues [65]. This suggests that SecF interacts with YidC using an electrostatic mechanism. While the periplasmic region of YidC is important for the protein to interact with SecDFYajC, notably YidC is still able to function efficiently with Sec-dependent substrates even with this region deleted.

5. The mitochondrial Oxa1 translocase

As described in previous sections, the mitochondrial Oxa1 plays a critical role in the insertion and assembly of the energy-transducing respiratory complexes [1,33]. Disrupting the Oxa1 function in *Saccharomyces cerevisiae* leads to a complete deficiency in complex IV (cytochrome *c* oxidase) and a marked decrease in the amounts of the respiratory complexes III (bc1) and V (F1Fo ATP synthase) [1,2,6]. This coincides with a strong reduction in inner membrane levels of the mitochondrial-encoded subunits Cox1 and Cox2 (cytochrome *c* oxidase) and a partial reduction in the mitochondrial encoded Atp4 and Atp6 (F1Fo ATP synthase), and cytochrome *b* (cytochrome *c* reductase) in the inner membrane [33,38]. These studies and further cross-linking experiments showed Oxa1 is important for the membrane insertion and directly mediates the cotranslational insertion of proteins synthesized in the mitochondria [33].

Surprisingly, the reduction of Oxa1 in human mitochondria by RNA interference (RNAi) did not decrease (or possibly increase) the level of complex IV [88]. However, there was a strong reduction in the level of complex V and a decrease in complex I (NADH reductase). In humans, the role of Oxa1 in the membrane assembly of NADH reductase may have to do with the fact that the human mitochondrion encodes six of the subunits of NADH reductase. The human Oxa1 may therefore play a critical role for the insertion of the mitochondrial-encoded subunits of the NADH reductase complex. Interestingly, studies in bacteria have shown that the bacterial YidC is required under anaerobic conditions and this may have to do with its role in the assembly of NADH dehydrogenase I. The level of this complex was reduced as YidC was depleted, in particular subunit K (NuoK) of the protein complex [72].

In mitochondria, Oxa1 facilitates the insertion of mitochondrial-encoded proteins and certain nuclear-encoded proteins that are imported into the mitochondria and integrated into the inner membrane [30,32,36]. It acts as a translocase for the translocation of the N-tail of Cox II, a protein that contains two transmembrane segments with an N-out and C-out topology [35] (Fig. 4). Cox II is synthesized in a precursor form with an amino-terminal signal peptide. Insertion and processing of Cox II require Oxa1 [30,32] and the electrical potential across the inner membrane [33,35]. In this pathway, Oxa1 interacts with Cox II in a cotranslational manner and is believed to function not only as a translocase but also as a ribosome receptor. After insertion, the N-terminal signal peptide is removed by proteolytic cleavage by Imp1p [64]. Translocation of the large carboxyl-terminal domain of CoxII requires Oxa2 (also called Cox18) and is also facilitated by the Pnt1p and Mss2p proteins (Fig. 4) [8,31,79]. Showing that Oxa2 plays a critical role, *oxa2* (*cox18*) knockout strains in *S. cerevisiae*, *Schizosaccharomyces pombe*, and *Neurospora crassa* lead to a strong reduction in complex IV with no affect on complex V [19,21,86]. These results suggest that Oxa2 plays a specialized role in the membrane biogenesis of complex IV.

In order for Oxa1 to function in cotranslational insertion of CoxII, the protein needs to contain the C-terminal matrix exposed domain. This domain is essential for the ability of Oxa1 to bind to ribosomes [37,91] and cross-linking studies have shown that Oxa1 interacts with the ribosomal protein Mrp20 within the large ribosomal subunit. Therefore, Oxa1 can act directly as a ribosome receptor, in contrast to Oxa2 which typical lacks the long matrix exposed domain.

In contrast to the insertion role in CoxII biogenesis, Oxa1 plays primarily an assembly role at a post-insertion step for the F1Fo ATP

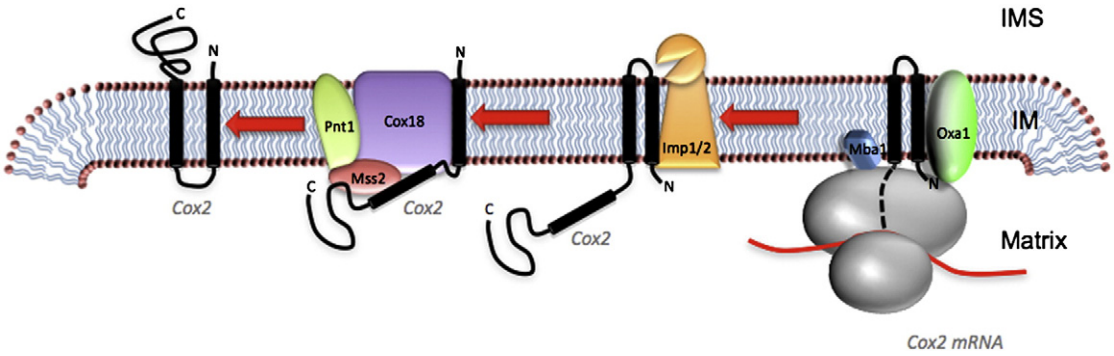

Fig. 4. The role of Oxa1 and Oxa2 in the membrane biogenesis of the mitochondrial Cox2 protein. Cox2 is synthesized in a precursor form with an N-terminal signal peptide. The precursor protein is cotranslationally targeted to the membrane via the interaction of the ribosome–Cox2 nascent chain complex with Oxa1 and Mba1. After Oxa1 mediated the insertion of the N-terminal region of Cox2, Imp1 (of the Imp1/2 complex) cleaves off the N-terminal signal peptide. The large C-terminal domain of Cox2 is subsequently translocated across the membrane by Oxa2 (Cox18) together with Pnt1 and Mss2.

synthase complex (Fig. 5). The ATP synthase complex is composed of a membrane-embedded Fo domain and a peripheral F1 domain. The Fo sector contains the integral membrane protein subunits Atp9 (subunit c in bacteria), ATP6 (subunit a in bacteria), Atp8 (subunit b in bacteria), and Atp4 and a few other subunits (not found in bacteria) that generates a functional proton-conducting Fo channel. As expected, under Oxa1 knockout conditions, there was a strong reduction in the amount of the ATP synthase complex [1,91]. Surprisingly, most of Atp9 oligomer was found to form a complex with the F1 subunits, but its further assembly with the Fo Atp6 subunit was disrupted [38]. Similar results were found when both the *oxa1* and *oxa2* genes were knocked out. Stuart and coworkers suggest that their data supports a model where Oxa1 is required for the correct assembly of the Atp9 oligomer and Atp6 into the F1F0 ATP synthase complex. In their study, they also found that Oxa1 forms a stable complex with the Atp9 oligomer [38] and can be copurified with the intact F1Fo ATP synthase. Interestingly, Oxa1 does not require the C-terminal ribosome binding domain to interact with newly synthesized Fo Atp9 or to copurify with the Atp9 oligomer or F1Fo ATP synthase [38]. The combined data suggest that Oxa1 functions in a postinsertion role with the Atp9 oligomer and ATP synthase complex. In bacteria, YidC is found to interact with the Foc oligomer and can be copurified with the F1Fo ATP synthase [76,103].

Like the bacterial YidC, extensive mutagenesis has been performed on the mitochondrial Oxa1 protein (see [7,55]). Mutations that impair activity have been isolated in the intermembrane space (IMS) loops, the transmembrane segments and two of the matrix exposed loops [55]. Interestingly, mutations in TM1, TM2, and TM5 affected the assembly of complex III, IV (cytochrome *c* oxidase), and V (F1Fo ATP synthase). Also, deletion of the entire C-terminal matrix-exposed domain led to strong defects in assembly of complex IV but no effect on complex V assembly. This deletion mutant led to stringent growth defects on nonfermentable carbon sources [91]. The mutations in the IMS loops showed different effects on the assembly of complex IV and V. The F229S–E65G mutant was affected in the assembly of both complex IV and V, while the L240S mutant was affected only in complex IV. Genetic evidence for functional interactions between various domains in the Oxa1 protein was determined by isolating intragenic suppressor mutations that restored respiration starting with their respiratory defective mutants. This analysis provided genetic evidence of TM2–TM5 and TM4–TM5 and IMS loop 2–TM4 interactions.

How do the structure–function studies on the mitochondrial Oxa1 relate to the studies on the bacterial YidC? Both structure–function studies with the mitochondrial Oxa1 and the bacterial YidC show that TM segments 1, 2, and 5 (TM 2, 3, and 6 in the *E. coli* YidC) are important. However, the genetic suppressor evidence showing functional interactions between TMs are quite different. Therefore, it is vital to determine the structure and transmembrane helix packing of the Oxa1/YidC protein members to understand the folding of the protein. The *N. crassa* Oxa1 has been reported to be a tetramer in detergent [61] although it appears to be a dimer when bound to translating ribosomes [48], as observed with the bacterial YidC.

Remarkably, in *S. cerevisiae*, there is strong evidence for an undiscovered alternative pathway for insertion into the mitochondrial inner membrane. First, Oxa1 is not absolutely essential for the formation of the ATP synthase. A small amount of the ATP synthase is still formed

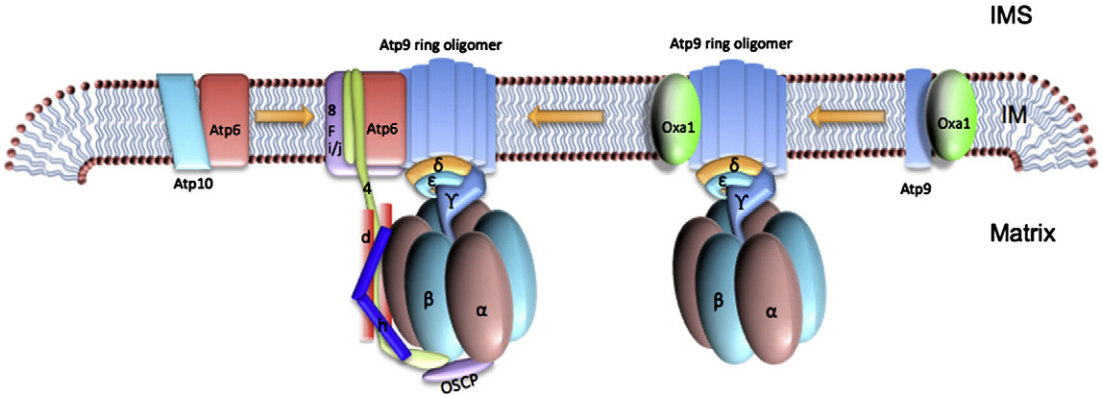

Fig. 5. The assembly role of Oxa1 in the formation of the mitochondrial F1Fo ATP synthase. First, Oxa1 is proposed to mediate the assembly of the Atp9 (subunit 9) ring since it copurifies with the Atp9 monomer and Atp9 ring. Second, Oxa1 keeps Atp9–F1 complex in a competent state so that Atp6 (subunit 6) can be assembled into the final complex. The Fo sector contains the Atp9 oligomer, Atp6, Atp8 (subunit 8), subunit f, and i/j. The F1 sector contains the α, β, γ, δ, and ε subunits. There is also a peripheral stalk composed of subunits 4, d, h and OSCP.

when *oxa1* was knocked out [1,38]. Second, the Atp9 oligomer (corresponding to the Foc oligomer in bacteria) was still formed in normal or slightly reduced amounts in the Δ*oxa1* strain, demonstrating that Oxa1 is not required for insertion and oligomerization of Atp9 in mitochondria. This is in contrast to the bacterial system where YidC is required for the insertion/oligomerization of subunit c [103]. Third, the insertion of cytochrome *b*, Atp4 and Atp6, though impaired, still occurs in the *Δoxa1* strain. Fourth, mutations in the *S. cerevisiae* cytochrome *c*1 compensates for the absence of Oxa1 [28]. Fifth, the absence of mitochondrial AAA protease restores the accumulation of the Fo ATP synthase subunits (Atp6, Atp4) in the *S. cerevisia oxa1* knockout strain [52].

6. The chloroplasts Alb3 system

Alb3 plays an important role for integration of LHCPs into thylakoid membranes (Fig. 6; right side) [57,110]. LHCPs are the most abundant proteins in thylakoid membranes where they form the light harvest antennas in the photosystems. The Alb3-dependent LHCPs are known to use the SRP system for targeting [47,53,94]. However, several other tested membrane proteins such as PsbW, PsbS, PsbY, and PsbX that do not require SRP for targeting, do not require Alb3 or the other known protein translocase systems for insertion into the thylakoid membrane [110]. This suggests for chloroplast membrane biogenesis, the main role of Alb3 may be connected to its specialized function in the posttranslational insertion of cpSRP-dependent membrane proteins.

The Alb3-dependent LHCPs are nuclear encoded proteins that are imported from the cytoplasm into the chloroplast stroma compartment. After removal of the chloroplast targeting sequence, LHCP forms a soluble transit complex with the cpSRP (Fig. 6; right side) [81]. The cpSRP is unusual in that it does not contain an RNA component that is found in the cytoplasmic SRP in bacteria and the ER [54]. Rather, it contains a novel protein component called SRP43 that is critical in the posttranslational targeting pathway [81]. SRP43 has an interesting structure that contains three chromodomains and four ankyin repeats [87]. The SRP43 recognition element within LHCP is an 18 amino acid hydrophilic motif (L18) between transmembrane segments 2 and 3 [13]. The LHCP L18 domain is specifically recognized by the cpSRP43 protein [95]. The LHCP–cpSRP54–cpSRP43 transit complex is then targeted to the thylakoid membrane by its interaction with cpFtsY, the SRP receptor (Fig. 6) [27,81]. Interestingly, at the membrane, cpSRP–FtsY forms a complex with Alb3. Moore et al. [58] showed each of these components can be precipitated using affinity tags. More recently, the nature by which Alb3 recruits cpSRP to the membrane has been investigated. The C-terminal domain of Alb3 is able to bind to the cpSRP43 component of the cpSRP complex [18]. The chromodomains 2 and 3 in cpSRP43 are needed for Alb3 binding with chromodomain 2 playing a central role as the binding platform.

It is very likely that Alb3 family members play additional roles in thylakoid membrane protein biogenesis. Similar to the bacterial system, Alb3 interacts with the Sec translocase, as it can be cross-linked to cpSecY [45]. Therefore, Alb3 may assist in the membrane biogenesis of Sec-dependent proteins, although there is no direct evidence for this function. Alb3 has been implicated in the folding and assembly of the D1 protein into PSII (see below). The D1 protein is a chloroplast-encoded protein with five transmembrane segments. It is synthesized by chloroplast ribosomes and interacts with cpSRP54, but not cpSRP43, during synthesis (Fig. 6; left side) [62]. The D1 protein is inserted into the thylakoid membrane by cpSecY, as demonstrated with cross-linking and coimmunoprecipitation experiments which showed that nascent chains of D1 interact with the cpSecY, and subsequently assembled into PSII [120]. However, it was shown in the *Chlamydomonas reinhardtii alb3* knockout strain, where the PSII system is perturbed, that although D1 can still be integrated into the membrane, without the proper function of Alb3, it is not efficiently assembled into the PSII complex [26]. These data and others [66] suggest that Alb3 homologs may act as a chaperone or assembly factor for the D1 protein. However, more work is needed to definitively prove this.

In *Arabidopsis*, the other Alb3 homolog called Alb4, cannot compensate for the deletion of the *alb3* gene as the *alb3* knockout strain leads to a severe reduction in the amount of chlorophyll content, an unusual chloroplast appearance and a seedling lethal phenotype [90]. An *alb4* knock down in RNAi-treated *Arabidopsis* produces a less severe phenotype although the chloroplast has an altered structure. They are more spherical and larger, and the grana stacks are not pressed as closely together as those in wild-type strain [23]. These studies with the Alb3 and Alb4 *Arabidopsis* mutants indicate that the Alb homologs are important for chloroplast membrane protein biogenesis, but have different functions. Recently, Benz et al. [5] have analyzed the *alb4* knock down strain in more detail and found that the assembly of the F1Fo ATP complex is affected. The ATPase activity is also reduced to some extent, and Alb4 interacts with the CF1β and CFoII ATP synthase subunits [5]. The conclusion from this study is that Alb4 plays a role in the assembly and stabilization of the F1Fo ATP synthase complex.

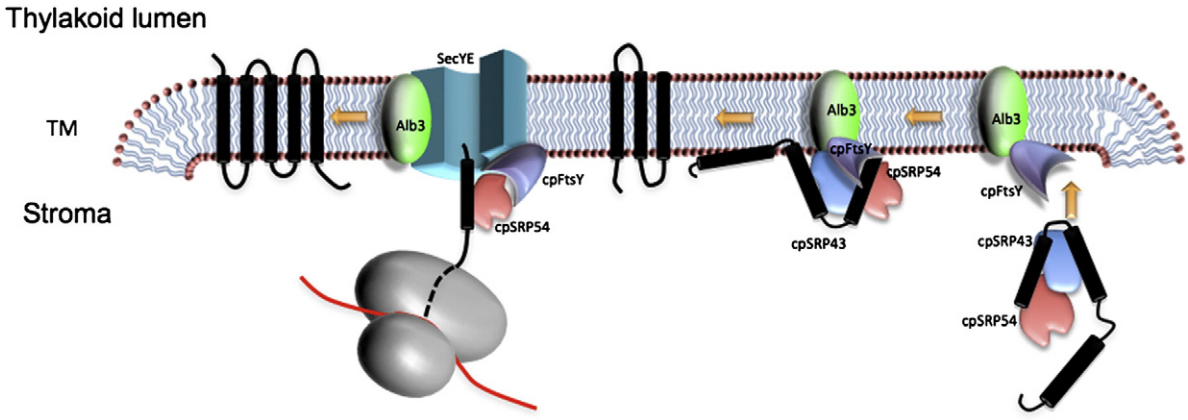

Fig. 6. Function of Alb3 in the biogenesis of chloroplasts thylakoid proteins. SRP-dependent LHCP proteins require Alb3 for membrane integration (right). Alb3 has been proposed to play also a role for Sec-dependent proteins to be cotranslationally inserted into the thylakoid membrane (left). LHCP, after it is imported into the chloroplast stroma and has had its targeting sequence removed, forms a transit complex with cpSRP43/cpSRP54. The interaction of cpSRP with LHCP occurs via the cpSRP43 component which binds to the L18 motif between TM2 and TM3 of LHCP. Both Alb3 and cpFtsY interact with LHCP–cpSRP43/cpSRP54 complex. GTP hydrolysis by both SRP54 and FtsY results in the release of LHCP from cpSRP, allowing Alb3 to insert LHCP into the thylakoid membrane. cpSRP then recycles to the stroma where it can be used again in membrane targeting.

7. Conclusion

In the past few years, there have been important advances on the role of the YidC/Oxa1/Alb3 family of proteins in the assembly of respiratory- and energy-transducing complexes. This universally conserved family is important for the formation of the F1Fo ATP synthase in chloroplasts, mitochondria and bacteria. However, there can be specialized functions depending on the particular system under study. In bacteria, for example, YidC plays a critical role in the membrane insertion of several subunits of the Fo complex. However, in mitochondria, Oxa1 is not absolutely needed for the insertion of the Fo subunits into the mitochondrial inner membrane. Indeed, the insertion of the Foc subunit or formation of the Foc oligomer is not strongly affected in an *oxa1* knockout mutant. In chloroplasts, Alb3 seems to play a specialized role in the insertion of the SRP-dependent LHCP proteins into the thylakoid membrane and is needed for efficient assembly of the D1 protein into photosystem II.

Another recent advance in the YidC area is the cryoelectron microscopy model showing YidC forms a dimer on translating ribosomes with substrate at the interface region between the 2 YidC monomers. The model, although at low resolution, provides a mechanism by which YidC can bind substrate and allow the substrate to partition into the lipid bilayer. However, it is vital to test this proposed structure of YidC as models based on cryo-EM can sometimes be inaccurate.

Although there has been progress in the YidC/Oxa1/Alb3 field, many questions remain. What is the molecular basis of how this family of proteins can function as an insertase, translocase, chaperone, and assembly factor? Do these proteins function as a dimer or monomer when acting as an insertase for substrates that are not bound to the ribosome? Similarly, in bacteria, is the oligomeric state of YidC similar when it functions cooperatively with the SecYEG protein-conducting channel? What are the structural features of a protein that make it dependent on YidC for membrane prot\ein insertion? In the chloroplast system, do Alb3 and Alb4 function in cooperation with the Sec machinery as has been seen in the bacterial system? What are all the substrates of this family of proteins in bacteria, mitochondria, and chloroplasts? In the mitochondrial and chloroplasts system, can the Oxa1/Alb3 family members function on their own as insertases? A high-resolution structure of YidC family members is essential to understand how substrates are inserted into the membrane and how they dissociate from the chaperone to integrate into the lipid bilayer.

We expect that future studies will deepen our understanding of the contribution of the YidC/Oxa1/Alb3 family of proteins to membrane protein biogenesis and shed light on the mechanism by which these proteins promote membrane protein insertion and help fold membrane proteins within the membrane interior.

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