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Minireview

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The YidC/Oxa1/Alb3 protein family: common principles and distinct features

Abstract: The members of the YidC/Oxa1/Alb3 protein family are evolutionary conserved in all three domains of life. They facilitate the insertion of membrane proteins into bacterial, mitochondrial, and thylakoid membranes and have been implicated in membrane protein folding and complex formation. The major classes of substrates are small hydrophobic subunits of large energy-transducing complexes involved in respiration and light capturing. All YidC-like proteins share a conserved membrane region, whereas the N- and C-terminal regions are diverse and fulfill accessory functions in protein targeting.

Keywords: bacteria; biogenesis; membrane protein insertion; mitochondria; targeting; thylakoids.

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Introduction

Biological membranes of living cells form hydrophobic barriers that separate the intracellular space from the extracellular environment and allow the maintenance of distinct cellular compartments. Many essential cellular processes depend on the function of membrane proteins that are anchored to the membrane by one or more transmembrane segments (TMSs). The biogenesis of membrane proteins is an important but still largely unresolved topic in biology. In bacteria, most membrane proteins are targeted to the membrane as nascent chains by the signal recognition particle (SRP) and partition into the lipid bilayer via the conserved Sec translocon (reviewed in du Plessis et al., 2011). In recent years, it has become apparent that not all membrane proteins follow this route. A variety of small hydrophobic proteins, initially believed to insert spontaneously without the aid of proteinaceous factors (Geller and Wickner, 1985; Kiefer and Kuhn, 1999), were found to be inserted by YidC. YidC is a member of the YidC/Oxa1/Alb3 protein family that is present in all three domains of life. These insertases are involved in various functions, including membrane protein insertion, folding, and assembly (Dalbey et al., 2011; Funes et al., 2011). Here, we summarize and discuss the current insights on the structure and function of YidC-like proteins from different organisms/organelles. The review focuses on the common principles of YidC-mediated membrane insertion and emphasizes the distinct features within the protein family.

Phylogenetic distribution

The YidC/Oxa1/Alb3 protein family is widely spread throughout all three domains of life (Yen et al., 2001). Most bacterial and eukaryotic genomes encode at least one YidC protein. In the archaeal domain, YidC proteins are present in some species within the Euryarchaeota but absent in Nanoarchaeota and Crenarchaeota (Pohlschroder et al., 2005; Zhang et al., 2009). Most bacteria contain only one copy, but Gram-positive bacteria, such as Bacilli, Lactobacilli, Actinobacteria, and some Clostridia, often harbor two YidC homologs (Funes et al., 2009, 2011; Zhang et al., 2009). The number of YidC homologs in eukaryotic cells/organelles varies between different organisms with a maximum number of six in the plant *Arabidopsis thaliana*. In contrast to the bacterial systems, the YidC homologs of eukaryotes are not found in the cytoplasmic membrane but only in the thylakoid membrane of chloroplasts (Alb proteins) and the inner mitochondrial membrane (Oxa proteins). A recent study (Zhang et al., 2009) suggested that Oxa and Alb proteins were derived from separate prokaryotic ancestors, but most likely not directly from Proteobacteria and Cyanobacteria, as one would assume based on the endosymbiotic theory. Additional separate gene duplications and/or secondary losses of Oxa/Alb proteins led to a rich diversity and to the distinct numbers of homologs found in each organism. For example, mitochondriate protozoa that contain no canonical mitochondria, but degenerate mitochondria without respiratory complexes (mitosomes or hydrogenosomes; van der Giezen and Tovar, 2005), lack genes encoding for Oxa homologs but also lack known Oxa substrates such as Cox2, Cox3, or subunits of the F_1F_0 ATP synthase (Preuss et al., 2005; Reif et al., 2005). Likewise, no Alb homologs are encoded in *Plasmodia* or Oomycetes, which possess degenerate plastids without light-harvesting complexes or appear to have secondarily lost plastids, respectively (Cavalier-Smith, 2000; Macasev et al., 2000). These data show that duplication/losses of these genes occurred multiple times during evolution and pose the interesting question whether the loss of YidC-like insertases preceded the loss of its substrates or the other way around.

In organisms (or organelles) with multiple YidC homologs, there is functional differentiation. For example, the *A. thaliana* Alb3 plays an essential role in the assembly of chlorophyll-containing photosynthetic complexes (e.g., photosystems I and II; Wang and Dalbey, 2011) and is involved in the posttranslational integration of light-harvesting chlorophyll-binding proteins (LHCPs) into thylakoid membranes (Moore et al., 2000; Woolhead et al., 2001). In contrast, Alb4 appears to be closely related to yeast Oxa1 and *E. coli* YidC and may be involved in the biogenesis of the CF_1CF_0 ATPase (Benz et al., 2009). In the bacterium *Bacillus subtilis*, the expression of either YidC homolog SpoIIIJ and YqjG is sufficient to sustain vegetative cell growth (Saller et al., 2009). Whereas YqgJ has a specific function in the genetic competence development (Saller et al., 2011), SpoIIIJ is specifically required for spore formation (Errington et al., 1992; Serrano et al., 2003; Saller et al., 2009). In *Streptococcus mutans*, the deletion of YidC1 does not result in an obvious phenotype, whereas the deletion of YidC2 causes a stress-sensitive phenotype resembling that observed with SRP pathway mutants (Hasona et al., 2005) and competence defects (Funes et al., 2009).

Functional conservation

YidC-like proteins play a universally conserved role in the biogenesis of subunits of large energy-transducing complexes and its members are remarkably exchangeable among different organisms. For instance, the respiratory defects of yeast *oxa1*-null mutants can be rescued by the human and *Neurospora crassa* Oxa1 (Bonnefoy et al., 1994a; Nargang et al., 2002) and *Escherichia coli* YidC (Preuss et al., 2005). In turn, *E. coli* YidC function can be complemented by Alb3 or Alb4 (Jiang et al., 2002; Benz et al., 2009), the YidC homolog from the photosynthetic bacterium *Synechocystis* sp. PCC6803 (Slr1471; Sven et al., 2008), and either yeast Oxa1 or Oxa2/Cox18 (Preuss et al., 2005; van Bloois et al., 2005, 2007). However, specific differences can also be observed. For example, the depletion of the *E. coli* YidC and yeast Oxa1 results in reduced amounts of assembled F_1F_0 ATP synthase and cytochrome *o* oxidase (Hell et al., 2001; Stuart, 2002), whereas the human Oxa1 is involved in the biogenesis of the F_1F_0 ATP synthase and NADH dehydrogenase but has no effect on cytochrome *c* oxidase activity (Stiburek et al., 2007). In contrast, in *N. crassa*, Oxa1 depletion affects the levels of cytochrome *c* oxidase as well as NADH dehydrogenase (Nargang et al., 2002).

Structure and domain function

Despite their functional conservation, YidC/Oxa1/Alb3 proteins share only a low level of primary sequence similarity. Not a single amino acid is conserved throughout the entire protein family, and the length varies over a fourfold range (~200–800 amino acids; Yen et al., 2001; Jiang et al., 2003). All members have a conserved hydrophobic core region consisting of five TMSs (Figure 1) connected by short hydrophilic loops; however, experimental validation of this model was done for *E. coli* YidC only (Saaf et al., 1998). The core domain is composed of about 200 amino acids and, together with a short α-helical stretch directly preceding the core region, is sufficient for functionality (Jiang et al., 2003). The core exhibits the highest homology within YidC-like proteins, particularly core TMS1, TMS2, and TMS5 (Yen et al., 2001). Point mutations within the first two core TMSs of *E. coli* YidC lead to a cold-sensitive growth phenotype and a decreased insertion activity (Jiang et al., 2003; Yuan et al., 2007). However, further mutational analysis revealed a large promiscuity of the core residues (Jiang et al., 2003). The importance of core TMS2 (TMS3 in *E. coli* YidC) is also evident from chemical cross-linking to known YidC substrates (Klenner et al., 2008; Yu et al., 2008) all across the membrane helix,

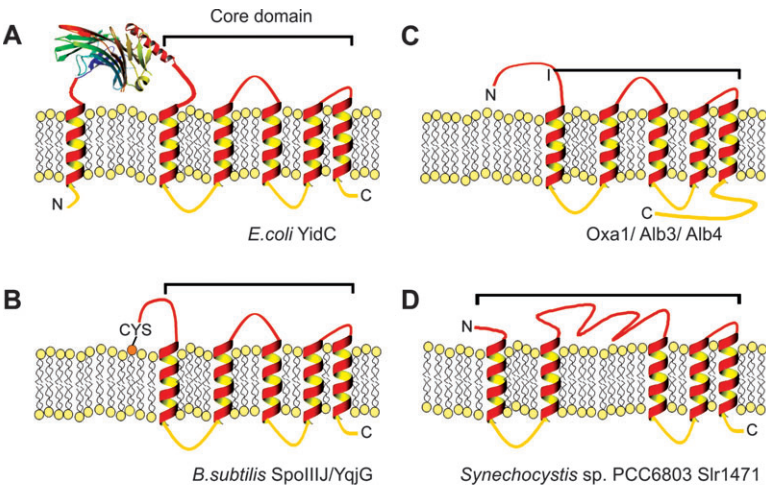

Figure 1 Topology models of YidC proteins from various organisms. (A) YidC from *E. coli*, including the resolved crystal structure of the large periplasmic domain (Oliver and Paetzel, 2008), (B) SpoIIIJ/YqjG from *B. subtilis*, (C) Oxa1 from *S. cerevisiae* and *A. thaliana* Alb3 and Alb4, and (D) Slr1471 from *Synechocystis* sp. PCC6803. The YidC *E. coli* model derives from experimental data (Saaf et al., 1998), and the remaining derives from sequence alignments and TMS prediction programs.

whereas core TMS4 (*E. coli* YidC TMS5) cross-links merely at the central and outer membrane face (Klenner and Kuhn, 2012). Additional substrate contact surfaces could be located at core TMS3 (*E. coli* YidC TMS4) and surprisingly in the noncore TMS1 of *E. coli* YidC (Klenner et al., 2008).

For some YidC homologs, the core structure seems to deviate from the general consensus. For example, the core of *Synechocystis* sp. PCC6803 (Slr1471) is predicted to contain a large periplasmatic domain between core TMS2 and TMS3 that is conserved within cyanobacteria and is essential for activity. This region possibly assists in substrate protein folding (Sven et al., 2008). The core domain of the YidC homologs of Euryarchaeota (Pohlschroder et al., 2005; Zhang et al., 2009) seems to comprise only three TMSs homologous to cores TMS1, TMS2, and TMS5. However, because functional studies on archaeal YidC-like proteins have not been performed thus far, it is uncertain if these proteins indeed specify a similar activity as YidC. Thus far, structural information on the core region is limited to a low-resolution structure derived from cryo-electron microscopy (cryo-EM) studies with two-dimensional crystals (Lotz et al., 2008). Interestingly, a 10 Å projection map reveals a low-density region in the YidC protein, which could exhibit a potential pore or crevice for membrane protein insertion.

The analysis of *E. coli* YidC indicates that, except for a short α-helical region preceding the core region, no domain outside the core is essential for the insertase activity (Jiang et al., 2003). The N- and C-terminal flanking regions of the core exhibit a variety in length, composition, and structure (Figure 1). In most Gram-negative bacteria, the N-terminal domain is composed of an additional TMS followed by a large periplasmatic loop. Recently, the crystal structure of the periplasmatic domain of *E. coli* YidC was solved (Oliver and Paetzel, 2008; Ravaud et al., 2008). It confirmed the α-helical conformation of the functionally essential region preceding the first core TMS and revealed a potential protein binding cleft composed of a β-supersandwich fold flexibly linked to the TMSs via α-helices. Surprisingly, the domain shows structural similarity to a galactose mutarotase from *Lactococcus lactis*, but a major share of this domain does not fulfill an essential function as it can be deleted from the YidC without compromising viability. The function of this domain may even be organism specific as YidC homologs of Gram-positive bacteria lack a large periplasmic domain between the N-terminal TMS and the core domain.

With respect to the C terminus, YidC homologs can be divided into two groups (Figure 1; Table 1). Whereas many homologs are devoid of a C-terminal extension, some contain an elongated, highly positively charged C-tail that has been implicated in ribosomes (Oxa1) or SRP (Alb3) binding as detailed below.

The functional oligomeric state of YidC homologs is still uncertain. Cryo-EM studies with two-dimensional crystals of *E. coli* YidC (Lotz et al., 2008) and with Oxa1 and YidC bound to translating ribosomes (Kohler et al., 2009) suggest that YidC forms (head to tail) dimers that are stabilized upon ribosome binding. Native electrophoresis of *E. coli* YidC (van der Laan et al., 2001) and chloroplast Alb3 (Dunschede et al., 2011) indicates the presence of monomers and dimers, whereas gel filtration chromatography of the *N. crassa* Oxa1 suggests that the protein

Name (organism)	Full-length protein				C terminus			
	Length (amino acids)	pI	Charges		Length (amino acids)	pI	Charges	
			Negative	Positive			Negative	Positive
SpoIIIJ (*B. subtilis*)	259	9.64	15	27	16	10.00	2	6
YqjG (*B. subtilis*)	275	9.57	13	24	17	10.00	1	4
YidC (*E. coli*)	548	7.70	43	44	16	11.07	2	7
YidC1 (*S. mutans*)	271	10.05	20	40	33	10.76	6	15
YidC2 (*S. mutans*)	310	10.25	13	41	61	10.76	5	19
Oxa1 (*S. cerevisiae*)	402	10.13	24	44	86	10.73	8	22
Oxa2 (*S. cerevisiae*)	316	11.25	10	36	7	8.59	0	1
Alb3 (*A. thaliana*)	462	9.08	42	48	110	9.43	22	26
Alb4 (*A. thaliana*)	499	7.14	55	55	163	5.72	34	30

Table 1 Overview of the charge distribution in various YidC proteins.
Amino acid sequences of the YidC proteins were obtained from the National Center for Biotechnology Information database. Protein sequence alignment was performed using 'Kalign 2.0' software, and the start of the C termini of the various YidC proteins was based on the alignment to the experimentally determined *E. coli* YidC topology (Saaf et al., 1998). The number of positively and negatively charged amino acid residues are indicated.

forms a tetramer in detergent solution (Nargang et al., 2002). Thus far, there is no evidence that such oligomers are functional entities.

Membrane protein insertion

The first proof for a role of the YidC/Oxa1/Alb3 protein family in membrane protein biogenesis originated from the work on cytochrome *c* oxidase (Cox2p) of the inner mitochondrial membrane. Oxa1p deletion resulted in severely diminished cytochrome levels (Bauer et al., 1994; Bonnefoy et al., 1994b) and the failure in translocation of the N terminus of Cox2p from the mitochondrial lumen to the intermembrane space (He and Fox, 1997; Hell et al., 1997, 1998). In 2000, two studies showed that YidC, a homolog of Oxa1, constitutes a conserved membrane protein insertion pathway in bacteria. YidC could be cross-linked to TMSs of nascent membrane proteins (Scotti et al., 2000) and was shown to fulfill an essential role in the membrane insertion of the small phage protein M13 procoat (Samuelson et al., 2000) and Pf3 (Chen et al., 2002). A firm proof for the hypothesis that YidC is an independent membrane insertase emerged from *in vitro* reconstitution studies showing that the purified YidC is the sole proteinaceous requirement for the insertion of Pf3 and the rotary subunit of the F_1F_0 ATPase, F_0c, which represented the first authentic *E. coli* substrate for the YidC-only insertion pathway (Serek et al., 2004; van der Laan et al., 2004).

Thus far, the number of substrates that have been shown to solely use YidC for membrane insertion is limited. In general, YidC-only substrates are small (~10 kDa), with high hydrophobicity and devoid of extensive hydrophilic loops. Known YidC-only substrates, such as M13 (Samuelson et al., 2000) and F_0c (Yi et al., 2003; van der Laan et al., 2004), exhibit a similar structure (Figure 2A). They consist of two TMSs that form a hairpin loop albeit with different topology [i.e., with a N_{in}-C_{in} (M13) or N_{out}-C_{out} conformation (F_0c)]. On the other hand, Pf3 coat protein consists of only one TMS. The 15-kDa mechanosensitive channel of large conductance (MscL) consists of one cytoplasmic helix and two TMSs that are connected via a short polar stretch in the periplasm and is oriented in an N_{in}-C_{in} conformation

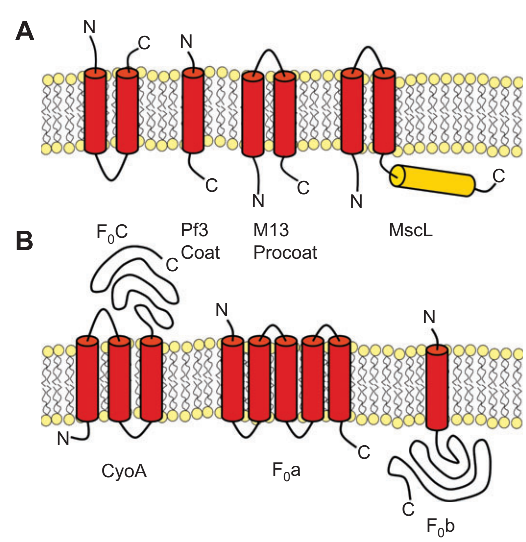

Figure 2 Topology models of (putative) YidC substrates. A number of membrane proteins insert via the YidC-only pathway (A), whereas others depend on both SecYEG and YidC (B).

(Chang et al., 1998). Contradictory results on its membrane insertion and oligomerization have been reported. Whereas one *in vivo* study demonstrated that MscL inserts in a YidC-dependent manner (Facey et al., 2007), another concludes that YidC is only required for homopentamerization of MscL (Pop et al., 2009). Two independent *in vitro* studies demonstrated the formation of functional homopentameric MscL pores in liposomes by conductivity measurements. Strong evidence was provided for the YidC-mediated insertion of MscL followed by oligomerization (Price et al., 2011), whereas, in the other study, MscL was shown to insert spontaneously in liposomes (Berrier et al., 2011). Because the latter study did not examine a requirement for YidC, one may argue that YidC would be needed for a high efficiency of insertion. A recent report using an *in vitro* approach suggested that the polytopic membrane proteins TatC and MtlA, which were previously considered Sec-exclusive substrates, could also be inserted via YidC alone albeit at very low efficiency (Welte et al., 2012).

The bacterial YidC and chloroplast Alb3 proteins also assist in the integration of membrane proteins that are targeted via the SRP pathway to the Sec translocon (Klostermann et al., 2002; du Plessis et al., 2006; Kol et al., 2009). Alb3 associates with the translocon by binding to chloroplast SecY (Klostermann et al., 2002), whereas the interaction between the Sec translocon and *E. coli* YidC appears to be mediated by the heterotrimeric SecDFYajC complex (Nouwen and Driessen, 2002). The interaction with SecDFYajC occurs via SecF and is strictly dependent on amino acids 215–265 (Xie et al., 2006) located in an exposed region of the large periplasmatic loop preceding the core domain of YidC (Figure 1). Because this region is not essential, the binding to the translocon might not be required for function per se. It has been suggested that only the Sec-independent function of YidC is essential and conserved (van Bloois et al., 2005), as Oxa1p can complement the YidC function in *E. coli*. YidC can be cross-linked to newly translated TMSs and is believed to function in the clearance or release of newly inserted TMSs from the central pore of the translocon (Scotti et al., 2000). Thus far, only cytochrome bo_3 oxidase subunit II (CyoA; du Plessis et al., 2006; van Bloois et al., 2006), F_1F_0 ATPase synthase subunit a (F_0a; Kol et al., 2009) and possibly subunit b (F_0b; Yi et al., 2003), and the NADH:ubiquinone oxidoreductase subunit K (NuoK; Price and Driessen, 2010) have been shown to require both YidC and SecYEG for insertion. Such proteins are generally larger with longer translocated hydrophilic loops than the YidC-only substrates (Figure 2B). CyoA and NuoK consist of three TMSs and contain a large periplasmic or cytoplasmic loop, respectively. F_0a contains five TMSs and is extremely hydrophobic due to the absence of long loop regions.

For the YidC-only substrates NuoK and M13 procoat, the YidC dependence is determined by the presence of negatively charged amino acid residues in the TMSs (Price and Driessen, 2010) and the extracellular connecting loop (Cao et al., 1995), respectively. With MscL, alternating the charges in the periplasmic loop rendered its insertion dependent on both SecYEG and YidC (Neugebauer et al., 2012), whereas, with F_0c, both the hydrophobic nature of the TMSs and the positive charges in the cytoplasmic loop were found to be crucial for YidC recognition, membrane insertion, and oligomerization (Kol et al., 2008). A recent genome-scale approach to find and categorize *E. coli* YidC substrates indicated that an unbalanced charge distribution renders a subset of membrane proteins dependent on YidC for membrane insertion (Gray et al., 2011). Interestingly, several proteins lose their dependency on YidC upon correction of the charge imbalance, whereas the YidC-independent protein may become YidC dependent on the perturbation of the charge distribution.

Recently, it was suggested that the YidC-dependent membrane protein insertion also involves YidD, a monotopic membrane protein that is highly conserved in Gram-negative bacteria (Yu et al., 2011a). The membrane levels of three YidC substrates (F_0c, the N terminus of CyoA and a M13-LepB hybrid protein) were reduced in an *E. coli yidD* knockout strain, whereas YidD was found to cross-link to nascent FtsQ, a membrane protein that contacts both SecYEG and YidC during membrane insertion. The *yidD* gene is, however, not essential.

Membrane protein folding and assembly

YidC-like proteins function not only as insertases but also as chaperones that assist in the folding and complex formation of newly inserted membrane proteins. Although the *E. coli* membrane protein lactose permease (LacY; Nagamori et al., 2004) and the maltose transporter (MalF; Wagner et al., 2008) insert into the membrane independently of YidC, their stability is decreased in cells depleted from YidC, suggesting a role for YidC in their folding. Oxa1 (Jia et al., 2007), Alb4 (Benz et al., 2009), and the *B. subtilis* SpoIIIJ and YqjG (Saller et al., 2009) copurify with the entire F_1F_0 ATP synthase, suggesting a role in complex assembly (e.g., the docking of the preassembled cytosolic F_1 domain onto the membrane-embedded F_0 domain). The last step in the biogenesis of the ATP synthase is the integration of F_0a into the complex; possibly, YidC-like proteins stall and stabilize the F_0a-lacking holocomplex until the insertion of F_0a. Interestingly, the release of the fully assembled complex from the YidC homologs seems to be crucial for functionality, as the F_1F_0 complex bound to SpoIIIJ or YqjG is devoid of ATPase activity (Saller et al., 2009). In *Chlamydomonas reinhardtii*, the insertion of the chloroplast-encoded protein D1 is mediated by cpSecY, whereas its functional assembly into photosystem II is strictly Alb3.1 dependent (Zhang et al., 2001; Ossenbuhl et al., 2004; Gohre et al., 2006). Also, Alb3.2, the other Alb3 homolog, fulfills an exclusive role in the assembly of photosystems I and II as shown by depletion studies (Bellafiore et al., 2002). Together, these studies indicate a conserved function of YidC proteins in the assembly of multimeric membrane complexes, but the detailed mechanism remains to be revealed.

Membrane quality control

Quantitative studies on the total membrane proteome of *B. subtilis* (Saller et al., 2011) and *E. coli* (Price et al., 2010; Wickstrom et al., 2011) provide a detailed insight in the effort of the cells to overcome the absence of SpoIIIJ/YqjG and YidC. YidC depletion results in the overexpression of proteins involved in chaperone-induced stress response, such as PspA in *E. coli* (van der Laan et al., 2003) and LiaH in *B. subtilis* (Jordan et al., 2006). In *E. coli*, YidC depletion also resulted in elevated levels of membrane-sequestered cytoplasmic chaperones, but this response was absent in *B. subtilis*. Recently, it was reported that *E. coli* YidC forms a complex with FtsH and HflK/C (van Bloois et al., 2008). This complex is thought to function in the quality control of membrane proteins in which YidC acts as chaperone and FtsH as protease that degrades misassembled membrane proteins (Ito and Akiyama, 2005). Although an increased level of FtsH was detected by proteomic analysis based on two-dimensional gel electrophoresis (Wickstrom et al., 2011), proteomic studies using metabolic labeling (Price et al., 2010; Saller et al., 2011) revealed no alteration of FtsH amount. Instead, HtpX, an FtsH homolog, which is believed to act in quality control (Shimohata et al., 2002), was found to be increased in both *E. coli* and *B. subtilis*. It would be of interest to determine whether these proteins also interact physically to confirm experimentally the presumed role of YidC in membrane protein quality control. The overexpression of the regulators of the glutamate-dependent acid resistance system, GadX and GadY, in an *E. coli* YidC depletion strain restores the ability to generate a proton motive force and leads to an increased membrane sequestering of the chaperone GroEL (Yu et al., 2011b). It was suggested that GroEL replaces YidC function and keeps newly synthesized membrane proteins in an insertion-competent conformation. In this respect, Oxa1 also appears not essential for mitochondrial insertion and assembly of the F_1F_0 ATPase complex as the defect in a Δ*oxa1* strain can be rescued by the deletion of the gene encoding the intermembrane space AAA protease Yme1p in *Saccharomyces cerevisiae* (Lemaire et al., 2000), further indicating a functional interaction between YidC-like proteins and the quality-control mechanisms in the membrane.

Protein targeting

The mechanism by which newly synthesized membrane proteins are targeted to YidC-like proteins may vary between homologs. The most detailed characterization of the targeting mechanism has been done for chloroplast Alb3, which not only assists in the cotranslational Sec-dependent integration of membrane proteins into the thylakoid membrane but also mediates the posttranslational insertion of LHCPs. LHCPs are synthesized in the cytoplasm as precursor proteins and imported posttranslationally into the stroma. After removal of the import sequence, they are bound by the chloroplast SRP (cpSRP) to form a soluble transit complex (Schuenemann et al., 1998) that is directed to the thylakoid membrane via association with the cpSRP receptor cpFtsY (Tu et al., 2000) and delivered to Alb3 (Moore et al., 2003). cpSRP consists of a 54-kDa subunit (cpSRP54) homologous to the 54-kDa SRP subunit found in prokaryotes and eukaryotes but is devoid of a SRP RNA. Instead, it contains a 43-kDa subunit (cpSRP43) that is unique to chloroplasts (Schuenemann et al., 1998). The transfer to Alb3 is mediated by the direct interaction of cpSRP43 with the positively charged, stromal-exposed C terminus of Alb3, which is intrinsically disordered and folds upon interaction with cpSRP43 (Tzvetkova-Chevolleau et al., 2007; Falk et al., 2010; Lewis et al., 2010). Two positively charged motifs (AKRS and SKRS) in the C terminus of Alb3 were found to be involved in this interaction (Falk et al., 2010), although the requirement for the SKRS motif has been challenged (Dunschede et al., 2011). Recently, an additional membrane-embedded cpSRP43 binding region was reported (Dunschede et al., 2011), which localizes to a part of TM5 that is oriented to the luminal side of Alb3 and cannot be accessed directly from the stroma.

In the inner membrane of the mitochondria, Oxa1 is responsible for the cotranslational insertion of mitochondrial-encoded proteins and posttranslational integration of nuclear-encoded proteins (He and Fox, 1997; Hell et al., 1997, 1998, 2001). Nuclear-encoded proteins are imported into the matrix by the TOM and TIM23 complex and subsequently inserted by Oxa1. For cotranslational insertion, translating mitochondrial ribosomes are recruited to Oxa1 via interaction of the large subunit with the highly positively charged α-helical C-tail of Oxa1 (Jia et al., 2003; Szyrach et al., 2003; Kohler et al., 2009). This interaction can be facilitated by the membrane-embedded receptor Mba1 (Preuss et al., 2001; Ott et al., 2006), which shows an overlap in function and substrate specificity to Oxa1p but does not seem to function in the same complex and can act independently (Preuss et al., 2001).

In *E. coli*, the insertion mediated by the YidC-only pathway also occurs cotranslationally (Chen et al., 2002, 2003; van der Laan et al., 2004), but YidC does not possess the extended highly charged C-terminal domain implicated in the Oxa1-ribosome interaction. Instead, it carries a short 16-amino acid C-terminal domain with a net positive charge (Table 1). Based on the observation that a solubilized C-terminally truncated YidC mutant did not coprecipitate with crude ribosomes, it was concluded that, also for *E. coli* YidC, the C terminus is important for ribosome binding (Kohler et al., 2009). However, it has not yet been addressed whether the C terminus is essential for YidC function *in vivo*. Remarkably, MscL is targeted to YidC in an SRP-dependent manner (Facey et al., 2007). Because SRP is involved in the targeting of nascent membrane proteins to the Sec translocase, it remains one of the unresolved questions how SRP functionally discriminates between YidC and Sec substrates.

Interestingly, in organisms with multiple YidC homologs, often, but not always, variants are present with and without the C-terminal extension. For example, the second mitochondrial YidC homolog, called Oxa2 or Cox18, lacks a C-terminal ribosome binding site (Preuss et al., 2005) and seems to play a role in posttranslational assembly of the cytochrome *c* oxidase (Souza et al., 2000). Also, the two YidC homologs of *S. mutans* differ in the presence of an elongated C terminus. Like Oxa1, the elongated C terminus of YidC2 is positively charged and has been implicated in ribosome binding (Funes et al., 2009; Table 1). Full-length YidC2, but not YidC1 or YidC2, lacking the C-terminal tail, supports cotranslational insertion into the mitochondrial inner membrane of *oxa1*-deficient *S. cerevisiae* mutants (Funes et al., 2009). Appending YidC1 with the YidC2 C terminus allows YidC1 to complement the stress sensitivity of a Δ*yidC2* strain (Palmer et al., 2012). However, in the case of the *B. subtilis* SpoIIIJ and YqjG, the C termini are both very short (~15 amino acids), and such a classification seems not apparent. In *A. thaliana*, both Alb3 and Alb4 contain large, highly charged C-terminal tails of 110 and 163 residues, respectively, whereas the Alb4 tail carries more negative charges (Table 1: pI=5.72) and positive charges outbalance (pI=9.43) in the cpSRP binding tail of Alb3 (Falk et al., 2010).

Concluding remarks and perspective

During the last decade, a multitude of new insights into the mechanisms of membrane protein insertion and assembly have been gained. This is particularly applicable for membrane protein insertion mediated by YidC and its homologs. Soon after the discovery of the novel YidC-only pathway, it became clear that this route is universally conserved and exchangeable between different organisms. Its importance is reflected by the fact that cells without YidC-like proteins encounter severe growth limitations or exhibit even a lethal phenotype. In general, all members of the YidC/Oxa1/Alb3 protein family share common functions in membrane insertion and assembly of energy-transducing complexes. However, each homolog also fulfills specific functions, which differ among organisms. In bacteria, the specific functions appear to be based on a common platform function in membrane protein insertion and assembly. The substrates are key proteins in a variety of cell-specific functions among other energy transduction, developmental processes such as sporulation and natural competence, and specific stress protection functions. Although, in some cases, specificity seems to relate to a requirement for cotranslation targeting, in general, the molecular basis of this specificity is still enigmatic, as the YidC/Oxa1/Alb3 protein family appears highly promiscuous considering its high degree of exchangeability in the different domains of life. A further understanding of the YidC function inevitably depends on a structural analysis of the YidC/Oxa1/Alb3 protein family in order to reveal the molecular principles of the insertase and chaperone function of these proteins.

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