Minireview

YidC/Oxa1p/Alb3: evolutionarily conserved mediators of membrane protein assembly

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Abstract This review focuses on a novel, evolutionarily conserved mediator of membrane protein assembly in bacteria, mitochondria and chloroplasts. This factor is designated YidC in *Escherichia coli*, and is localized in the inner membrane. YidC is homologous to Oxa1p in the mitochondrial inner membrane and Alb3 in the chloroplast thylakoid membrane, but does not seem to have a homologue in the endoplasmic reticulum membrane. It has been suggested that YidC operates both as a separate unit and in connection with the SecYEG-translocon depending on the substrate membrane protein that is integrated into the membrane. Mitochondria do not possess a SecYEG-like complex and Oxa1p is thought to form, or to contribute to the formation of, a novel translocon in the mitochondrial inner membrane. Alb3 in the chloroplast thylakoid membrane is, just like YidC and Oxa1p, involved in membrane protein assembly, but only few details are known.

Key words: Membrane protein; YidC; Oxa1p; Alb3; Translocon

1. Introduction

The biogenesis of inner membrane proteins (IMPs) in *Escherichia coli* is accomplished in three consecutive steps: (i) membrane targeting, (ii) membrane insertion and (iii) assembly and folding into the final, lipid-embedded and functional structure (reviewed in [1]). Defined sets of protein components have been identified that play a role in the first two stages. Most IMPs use the signal recognition particle (SRP) and its receptor FtsY for co-translational targeting in a process that resembles the targeting of secretory and membrane proteins to the endoplasmic reticulum (ER) in eukaryotic cells (reviewed in [2]). Recent genetic and biochemical evidence indicates that most IMPs insert at the translocon that was originally identified as the protein-conducting pore that receives and translocates secretory proteins targeted by the chaperone SecB (reviewed in [3]). Apparently, the SRP and SecB targeting routes converge at the membrane-embedded translocon that consists of the integral subunits SecY, SecE and SecG [4]. The peripheral subunit SecA is an ATPase that functions both as a receptor for SecB and as a molecular motor which drives the translocation of secretory proteins through the SecYEG protein-conducting pore (reviewed in [3]). In addition, SecA appears to be involved in the transfer of large periplasmic domains of IMPs, but is not required at the initial phase of membrane insertion [5,6]. In contrast, some small phage coat proteins have been reported to insert spontaneously into the membrane (reviewed in [7]) although this view has recently been challenged ([8]; see below). Very little is known about components involved in the final stages of IMP biogenesis: the release of transmembrane segments (TMs) from the translocon into the lipid bilayer, folding and acquisition of the correct topology, incorporation of cofactors, assembly into complex structures and quality control.

Both mitochondria and chloroplasts are believed to have a prokaryotic ancestor. Mitochondria contain two membranes: the outer and the inner membrane. Chloroplasts contain three different membranes: the outer, the inner and the internal thylakoid membrane. The inner membrane of mitochondria and the thylakoid membrane of chloroplasts resemble the *E. coli* inner membrane in many ways. Therefore, it is not surprising that there are similarities in the basic mechanisms of protein localization to the mitochondrial inner and chloroplast thylakoid membranes (reviewed in [9]). However, there are also notable differences which are in part related to the limited capacity of mitochondria and chloroplasts to synthesize their own proteins. Most proteins, including membrane proteins, are nuclear-encoded and have to be imported post-translationally into these organelles. This import involves the input of energy, the presence of an N-terminal targeting sequence on the imported protein, chaperones in the cytosol, translocation machineries in the outer and inner membranes, and a peptidase in the organelle lumen to remove the targeting sequence.

Recently, a novel and evolutionarily conserved factor has been identified that plays a key role in the assembly of inner membrane and thylakoid membrane proteins in bacteria, mitochondria and chloroplasts, respectively. This factor was initially discovered in mitochondria and designated Oxa1p.

2. Mitochondria

2.1. Protein targeting to the inner membrane in mitochondria

Targeting of nuclear-encoded proteins to the mitochondrial inner membrane follows three distinct pathways, which at first

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glance appear less related to targeting in bacteria (reviewed in [10]). First, solute carrier proteins are transported through the translocon in the outer membrane (the TOM complex) and transferred across the intermembrane space to a complex machinery (the TIM22 complex) that mediates insertion into the inner membrane. Second, certain IMPs follow the import route through the TOM complex but diffuse laterally from the general translocon in the inner membrane (the TIM23 complex) during import (stop–transfer mechanism). Third, some IMPs are first completely imported into the matrix before being re-inserted into the inner membrane (conservative sorting). This process requires the membrane potential and is thought to resemble the insertion of mitochondrially encoded IMPs.

Little is known about the targeting of mitochondrially encoded IMPs. Yeast mitochondria do not contain obvious SRP- or Sec-related proteins [11] and a targeting role for matrix chaperones has not been documented. Yet, a co-translational insertion mechanism would seem beneficial given the hydrophobic nature of IMPs. Indeed, a considerable fraction of the mitochondrial ribosomes is located at the inner membrane in both yeast and mammalian cells ([12] and references therein). The nature of the membrane association has not yet been fully elucidated although in yeast targeting of mRNA to specific inner membrane complexes might promote a co-translational insertion mechanism [13].

How do mitochondrial-encoded IMPs, and nuclear-encoded IMPs that are assembled via the conservative sorting mechanism, integrate into the inner membrane in the absence of a prokaryotic-type Sec-machinery? Recently, compelling evidence has been obtained suggesting that Oxa1p constitutes or contributes to a novel translocon for IMPs.

2.2. *Oxa1p is required for membrane assembly of a subset of mitochondrial IMPs*

The *oxa1* gene (encoding Oxa1p) was first isolated in yeast in a genetic screen designed to isolate components involved in strict oxygen-dependent growth [14]. Oxa1p was found to be essential for correct assembly of cytochrome *c* oxidase (oxa stands for oxidase assembly) and thus for respiration and growth on non-fermentable media [14,15]. Later, a more general role for Oxa1p in the assembly of both nuclear- and mitochondrial-encoded IMPs has been suggested based on the disturbed biogenesis of Oxa1p itself and the ATPase complex in *oxa1* mutant cells [16,17]. Oxa1p is a nuclear-encoded protein that is imported by virtue of a matrix targeting signal. The matured protein contains five (TM) segments and acquires an N_{out}–C_{in} orientation in the membrane with a large (ca. 90 amino acids) translocated N-terminal domain which has an overall negative charge [18].

In the first instance, studies on the effects of mutated *oxa1* focused on preCoxII (subunit of the cytochrome *c* oxidase) and preSu9 (subunit of the F_1F_0-ATPase) that, like Oxa1p, carry a large translocated N-terminal tail. The export of these tails seems to resemble the export of N-tails in prokaryotic systems [19]. They obey the 'positive-inside' rule of membrane topology and require a membrane potential for correct localization but are exported in a Sec-independent manner. Therefore, it has been suggested that Oxa1p and its prokaryotic homologue YidC are specifically involved in the biogenesis of N-tail proteins [16,20]. However, it has been demonstrated very recently that the role of Oxa1p in the assembly of IMPs is not restricted to N-tail proteins but extends to IMPs of various topology and complexity [21].

Strikingly, Oxa1p interacts directly with nascent IMPs that are mitochondrially encoded and with full-length IMPs that are nuclear-encoded [16,21]. This is consistent with a role for Oxa1p in both co-translational and post-translational insertion of IMPs into the membrane (Fig. 1).

3. Chloroplasts

3.1. *Protein targeting to the thylakoid membrane in chloroplasts*

Nuclear-encoded proteins destined for the thylakoid are synthesized with a bipartite targeting signal that consists of a stroma-targeting signal for import and a prokaryote-type signal peptide to mediate targeting to the thylakoid membrane (reviewed in [22]). An SRP-like complex (cpSRP) and a FtsY homologue (cpFtsY) have been identified that seem to be

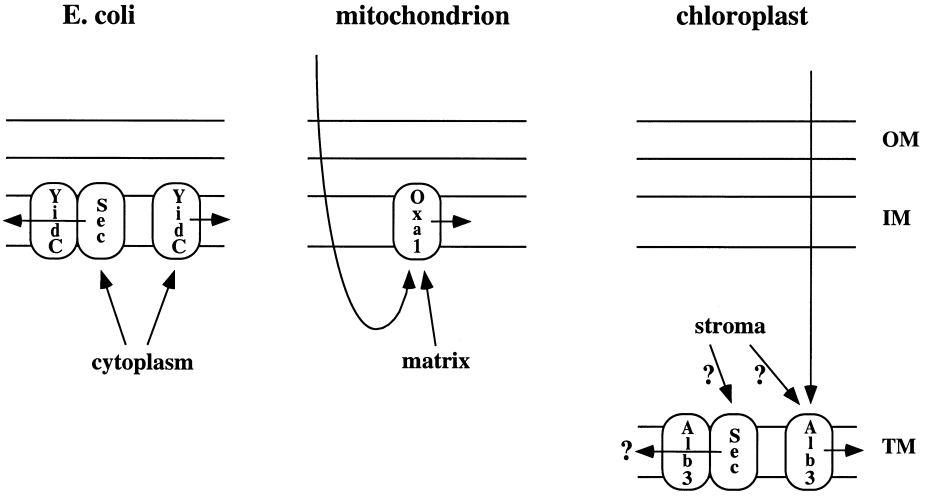

Fig. 1. Model for the roles of YidC, Oxa1p and Alb3 in membrane protein assembly. OM, outer membrane; IM, inner membrane; TM, thylakoid membrane. The involvement of Alb3 in the assembly of chloroplast-encoded IMPs and its connection with the Sec translocon is speculative.

essential for the targeting of a subset of nuclear-encoded thylakoid membrane proteins in a mechanism that is by nature post-translational. In addition, a co-translational interaction of the SRP with a chloroplast-encoded membrane protein has been shown [23]. Also, like in prokaryotes, a cpSecA-route and a 'spontaneous' insertion mechanism appear to be operational for specific membrane proteins. Genetic and biochemical data identified homologues of bacterial SecY and SecE in the thylakoid membrane of chloroplasts, suggesting the presence of a cpSec-translocon [24,25]. The connection of this translocon with targeting pathways and its role in membrane protein insertion has not yet been investigated in detail.

3.2. Alb3 is the chloroplast homologue of Oxa1p

The *alb3* gene (encoding the thylakoid membrane protein Alb3) was selected in a screen designed to isolate pigmentation-deficient *Arabidopsis* mutants (alb stands for albino) [26]. The Alb3-deficient chloroplasts showed abnormal morphology and very few thylakoid membranes. Recently, Alb3 has been implicated in the biogenesis of LHCP, a thylakoid membrane protein of N_{in}–C_{out} topology, based on antibody inhibition experiments [27]. LHCP is nuclear-encoded and requires cpSRP and cpFtsY for targeting to the thylakoid membrane [28–30]. Notably, membrane integration of LHCP is not inhibited by antibodies against cpSecY, suggesting targeting via cpSRP/cpFtsY to Alb3, although this needs to be substantiated by alternative assays [25] (Fig. 1).

4. YidC, *E. coli* homologue of Oxa1p, is involved in both Sec-dependent and Sec-independent IMP assembly

Originally, *E. coli* YidC was identified as an Oxa1p homologue of unknown function [15] that was later shown to be essential for viability [8]. Its topology appeared to be similar to that of Oxa1p except for the presence of an extra N-terminal signal–anchor sequence [31]. Using a site-specific photo cross-linking procedure, YidC was recently shown to interact with two nascent IMPs of different topology, Lep and FtsQ [8,32,33]. The observed interactions occurred early in biogenesis and appeared to be specific for the TM segments in the nascent polypeptides. Both Lep and FtsQ are targeted to the SecYEG-translocon via the SRP/FtsY route [4,32], suggesting a function for YidC in association with the translocon very different from Oxa1p and Alb3. Indeed, YidC can be co-purified with the Sec-translocon [33]. This suggests a physical connection although it is yet unclear which Sec component(s) is responsible for the interaction and whether the interaction is permanent or transient. Analysis of nascent FtsQ of different lengths revealed a sequential interaction of the TM first with SecY and then with YidC [34]. It has been proposed that YidC functions in mediating the transfer of TMs from the core translocon into the lipid bilayer [33]. Consistently, assembly of Sec-dependent IMPs, like Lep and FtsQ, is hampered upon depletion of YidC in vivo although the effect is relatively mild [8,34], suggesting that the assembly of these IMPs is not completely dependent on YidC. In contrast, assembly of the Sec-independent M13 procoat was almost completely blocked upon depletion of YidC [8]. Hence, YidC might function both in connection with the Sec machinery and in a Sec-independent mechanism that is apparently evolutionarily conserved in mitochondria and chloroplasts (Fig. 1). It should be noted that conditional depletion of an essential protein bears the risk of pleioptropic effects. In principle, depletion of YidC might affect essential membrane functions that indirectly influence the biogenesis of the IMPs that were analyzed.

No information is available on a possible involvement of YidC in the translocation of secretory proteins except that the in vivo effect of YidC depletion on secretion is mild and possibly indirect [8]. However, it cannot be excluded that YidC plays a role for instance in the evacuation of the signal sequence from the translocon. Eventually, in vitro reconstitution of Sec/YidC- and YidC-dependent assembly of IMPs is required to rigorously assess the function and substrate specificity of YidC. A first step towards this goal is the reconstitution of the initial membrane insertion of nascent FtsQ using proteoliposomes that contain SecYEG and YidC [35].

5. Inventory of the YidC/Oxa1p/Alb3 protein family

YidC homologues appear to be present in all eubacterial species but little is known on their structure and function. To identify members of the YidC protein family we carried out profile-based searches of the NCBI protein database using PSI-blast [36] and the hmmer program package (http://hmmer.wustl.edu). A selection of bacterial and organellar homologues found in this search is presented in Fig. 2 (for additional information see http://bio.lundberg.gu.se/febs/). In most eubacteria there is only one YidC homologue. However, we noted that species of *Bacillus*, *Listeria* and *Streptomyces* have two different homologues, as shown for *B. subtilis* and *B. halodurans* in Fig. 2. Disruption of one of the two *B. subtilis* homologues, SpoIIIJ, blocks spore formation at an intermediate stage but it is not clear if this is related to impaired assembly of general or spore specific membrane proteins [37]. Another observation is that a majority of eubacteria, including Proteobacteria, *Chlamydia*, *Aquifex*, and *Leptospira* seem to have an N-terminal periplasmic domain of approximately 300 amino acids that is lacking in the Firmicutes (gram-positive bacteria) (Fig. 2 and data not shown here). This may be related to the significant difference in membrane structure between the two categories of bacteria.

Also, for the organellar proteins there is apparently more than one member of the Oxa1p/Alb3 protein family in each organism. For instance, in *Schizosaccharomyces pombe* there are three different proteins that clearly belong to the YidC/Oxa1p/Alb3 protein family. In *Arabidopsis* there are six different forms of the protein. Nothing is known about the expression and substrate specificity of the different homologues. According to the TargetP prediction method [38], three of the *Arabidopsis* proteins are targeted to the chloroplast (numbered 3, 4 and 5 in Fig. 2) and three to the mitochondrion. For one of the latter forms there is experimental evidence that it is localized in the mitochondrial inner membrane and that it complements Oxa1p deficiency in yeast [39].

In the second and third rounds of PSI-blast we also noted a family of archaebacterial proteins distantly related to YidC/Oxa1p/Alb3. The predicted membrane topology of the archaebacterial proteins is to a large extent consistent with that predicted for eubacterial YidC (Fig. 2). However, as the extent of homology is rather poor it will have to be verified experimentally whether this family of archaebacterial proteins is functionally related to YidC.

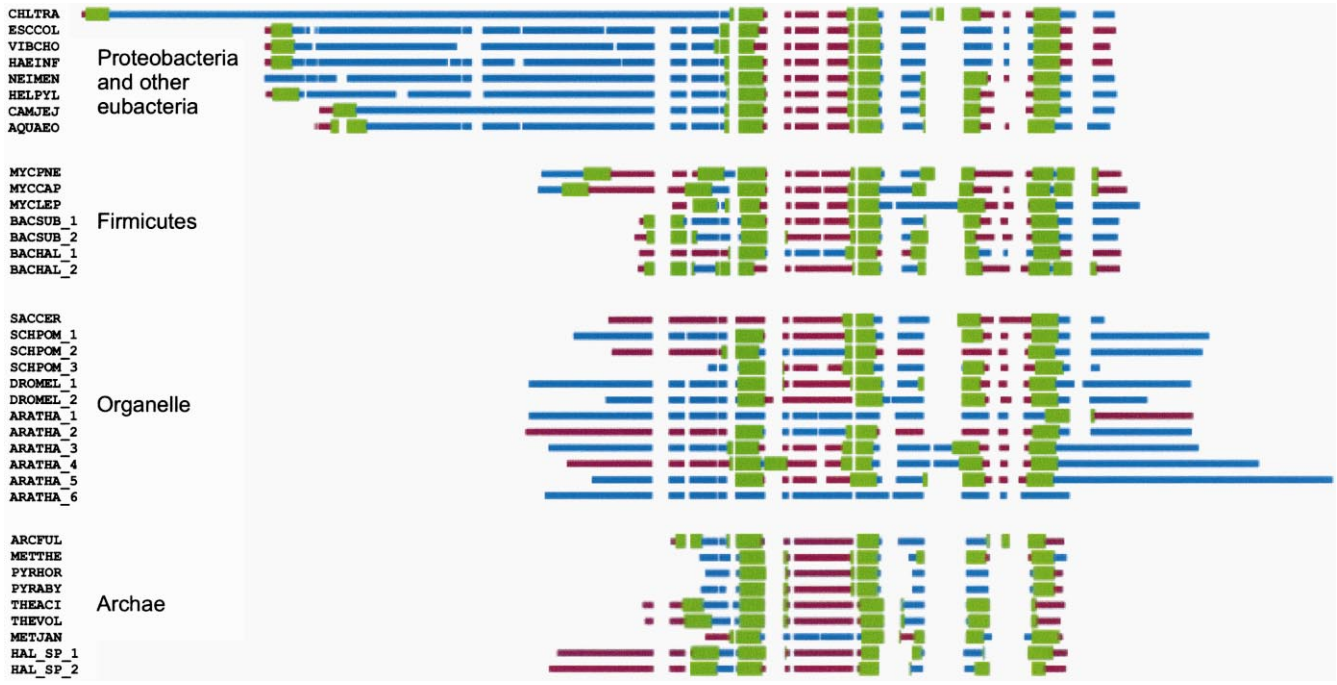

Fig. 2. Alignment and predicted membrane topology for selected YidC/Oxa1p/Alb3 homologues. A multiple alignment was created using CLUSTALW [47] and TM helices were predicted with TMHMM [48], generously provided by Anders Krogh, Copenhagen. In-house software was used to plot the results of TMHMM predictions on the multiple alignment template. Green rectangles indicate TM helices, blue and red lines are regions predicted to be outside and inside of the membrane, respectively. The organisms shown are *Chlamydia trachomatis* (CLATRA), *E. coli* (ESCCOL), *Vibrio cholerae* (VIBCHO), *Haemophilus influenzae* (HAEINF), *Campylobacter jejuni* (CAMJEJ), *Helicobacter pylori* (HELPYL), *Aquifex aeolicus* (AQUAEO), *Mycoplasma pneumoniae* (MYCPNE), *Mycoplasma capricolum* (MYCCAP), *Mycoplasma leprae* (MYCLEP), *Bacillus subtilis* (BACSUB), *Bacillus halodurans* (BACHAL), *Saccharomyces cerevisiae* (SACCER), *Schizosaccharomyces pombe* (SCHPOM), *Drosophila melanogaster* (DROMEL), *Arabidopsis thaliana* (ARATHA), *Archaeoglobus fulgidus* (ARCFUL), *Thermoplasma acidophilum* (THEACI), *Methanococcus jannaschii* (METJAN), *Methanococcus thermoautotrophicus* (METTHE) *Halobacterium* sp. NRC-1 (HAL_SP) *Pyrococcus horikoshii* (PYRHOR) and *Pyrococcus abyssi* (PYRABY). More information on the sequence alignment may be found at http://bio.lundberg.gu.se/febs/.

6. Complex formation of YidC/Oxa1p/Alb3

As mentioned above, *E. coli* YidC is in part associated with the Sec-translocon but also acts independently of the translocon. Notably, purified YidC has a tendency to dimerize in vitro [35] and cross-linking studies revealed the participation of YidC in high molecular weight complexes which remain to be characterized (Urbanus and Luirink, in preparation). Likewise, it has been claimed that Oxa1p forms part of an oligomeric complex although the size and composition of this complex have not been documented yet [16]. Nothing is known about a possible oligomerization of Alb3 or a connection with the cpSec-machinery.

7. Function of YidC/Oxa1p/Alb3

The YidC/Oxa1p/Alb3 family is required for the proper biogenesis of a subset of IMPs/thylakoid membrane proteins although the exact substrate specificity and the molecular basis for this specificity have not been determined. It has been suggested that Oxa1p, Alb3 and 'Sec-independent' YidC form part of a dedicated export machinery. However, effects of functional inactivation of Oxa1p (homologues) have been monitored using assays that define late stages in the biogenesis of IMPs, i.e. maturation, export of domains and biological activity. Therefore, it is not clear whether Oxa1p (homologues) functions in the reception of targeting complexes (including ribosomes), the insertion or assembly of TM domains in the lipid bilayer, the export of extramembranous domains, or the oligomerization of IMPs into complex structures.

Remarkably, *oxa1* mutants can be suppressed by mutations in *cyt1* [40]. The mutated cytochrome c_1 was proposed to prevent the degradation of subunits of respiratory chain complexes that are improperly integrated in the absence of Oxa1p. However, it is difficult to explain how mutated cytochrome c_1 can complement other adverse effects of inactivation of Oxa1p. Vice versa, Oxa1p suppresses respiratory defects caused by disruption of the genes encoding Rca1 and/or Afg3 [41]. Rca1 and Afg3 form part of a hetero-oligomeric proteolytic complex in the inner membrane that is involved in the degradation of unassembled subunits of IMP complexes such as the cytochrome *c* oxidase complex (reviewed in [42]). Together with another IMP, Yme1, Rca1 and Afg3 belong to a family of ATP-dependent proteases that also display chaperone activities. Possibly, Oxa1p cooperates with these factors to constitute a quality control mechanism for newly synthesized IMPs. Oxa1p might offer a protective environment for IMPs or assist their folding to increase stability. Consistent with this putative role for Oxa1p, it has recently been shown that both cytochrome *c* oxidase and ATPase complexes are rapidly degraded in a Δ*oxa1* mutant. Surprisingly, ATPase activity was restored in Δ*oxa1*/Δ*yme1* double mutants suggesting that the increased stability of ATPase subunits allows assembly of the ATPase. This implies that Oxa1p is not absolutely essential for functional assembly of the ATPase complex. FtsH is the *E. coli* representative of the ATP dependent

protease/chaperone family that is located in the inner membrane (reviewed in [43]). FtsH has been implicated in a variety of membrane-associated processes including assembly, topogenesis and degradation of IMPs such as SecY and YccA. FtsH is present in high molecular weight complexes that might be involved in the quality control of IMPs [44,45]. These complexes have not been fully characterized but they might contain YidC.

E. coli YidC seems unique among the Oxa1p homologues in that it plays an additional role in association with the Sec-translocon [33]. The sequential cross-linking of the FtsQ TM domain with SecY and Oxa1p is reminiscent of the early interactions of nascent Lep in the eukaryotic ER membrane that suggest insertion of the Lep TM at Sec61α (homologue of SecY) and subsequent diffusion towards TRAM [46]. It was suggested that TRAM plays a crucial role in the lipid partitioning of TM domains. Although YidC/Oxa1p/Alb3 and TRAM show no obvious sequence similarity, the resemblance in their interaction with nascent membrane proteins is suggestive of a shared, yet uncertain function.

Finally, it should be noted that the proposed functions of YidC, both independently of and in association with the translocon, are based on the analysis of only a limited subset of IMPs. Clearly, more IMPs of different topology and complexity need to be tested using these and other approaches to establish the generality and mechanism of YidC functioning.

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