Biochimica et Biophysica Acta 1793 (2009) 60–70

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Roles of Oxa1-related inner-membrane translocases in assembly of respiratory chain complexes

Nathalie Bonnefoy [a], Heather L. Fiumera [b], Geneviève Dujardin [a], Thomas D. Fox [b,*]

[a] Centre de Génétique Moléculaire, CNRS UPR 2167, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France
[b] Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853-2703, USA

ARTICLE INFO

Article history:
Received 19 March 2008
Received in revised form 2 May 2008
Accepted 5 May 2008
Available online 15 May 2008

Keywords:
Membrane insertion
Membrane translocation
Cytochrome c oxidase
ATP synthase
Mitochondrial ribosome
Cox18
YidC

ABSTRACT

Members of the family of the polytopic inner membrane proteins are related to *Saccharomyces cerevisiae* Oxa1 function in the assembly of energy transducing complexes of mitochondria and chloroplasts. Here we focus on the two mitochondrial members of this family, Oxa1 and Cox18, reviewing studies on their biogenesis as well as their functions, reflected in the phenotypic consequences of their absence in various organisms. In yeast, cytochrome c oxidase subunit II (Cox2) is a key substrate of these proteins. Oxa1 is required for co-translational translocation and insertion of Cox2, while Cox18 is necessary for the export of its C-terminal domain. Genetic and biochemical strategies have been used to investigate the functions of distinct domains of Oxa1 and to identify its partners in protein insertion/translocation. Recent work on the related bacterial protein YidC strongly indicates that it is capable of functioning alone as a translocase for hydrophilic domains and an insertase for TM domains. Thus, the Oxa1 and Cox18 probably catalyze these reactions directly in a co- and/or posttranslational way. In various species, Oxa1 appears to assist in the assembly of different substrate proteins, although it is still unclear how Oxa1 recognizes its substrates, and whether additional factors participate in this beyond its direct interaction with mitochondrial ribosomes, demonstrated in *S. cerevisiae*. Oxa1 is capable of assisting posttranslational insertion and translocation in isolated mitochondria, and Cox18 may posttranslationally translocate its only known substrate, the Cox2 C-terminal domain, *in vivo*. Detailed understanding of the mechanisms of action of these two proteins must await the resolution of their structure in the membrane and the development of a true *in vitro* mitochondrial translation system.

1. Introduction

The evolution of mitochondria as integrated eukaryotic cellular organelles required both the development of protein import machineries for the uptake of proteins synthesized in the cytoplasm, as well as machineries for the insertion of proteins into the inner mitochondrial membrane from the matrix compartment [1,2]. The first mitochondria presumably had the membrane insertion and translocase systems of their alpha-proteobacterial ancestors for the insertion of membrane proteins from the inside. Indeed, at least some extant unicellular eukaryotes retain remnants of the bacterial SecYEG translocase system [3]. However, in the well studied mitochondria of fungi, animals, and plants, there is no evidence for the presence of descendants of these bacterial translocase functions [4].

Polytopic inner-membrane proteins related to the *Saccharomyces cerevisiae* protein Oxa1, the first such protein discovered, represent one conserved function that facilitates insertion of mitochondrial inner-membrane proteins from the inner, matrix, side. Members of this ancient family of proteins have been shown to have critical roles in membrane insertion and assembly of energy-transducing complexes in eukaryotic organelles (mitochondria and chloroplast) and bacterial systems, although their precise mechanisms remain to be elucidated. In this review, we attempt to integrate experimental findings and information from comparative studies bearing on the functions and physiological roles of Oxa1-related proteins in mitochondrial biogenesis. Previous valuable reviews focusing on these proteins from a variety of perspectives include [5–12].

2. Amino acid sequence and evolutionary conservation of Oxa1-related proteins

The signature feature of mitochondrial Oxa1-related proteins is a group of five transmembrane (TM) domains that are likely to carry out its central function(s) in membrane protein insertion (Fig. 1) [2,11,13–16]. Spacing of the downstream four TM domains is highly conserved [17]. As expected for proteins with multiple hydrophobic segments, those Oxa1-related proteins that have been experimentally studied appear embedded within the inner-membrane of mitochondria by sub-fractionation and alkali treatment of mitochondria and have their C-termini located on the internal, *cis*, side of the membrane

* Corresponding author. Tel.: +1 607 254 4835; fax: +1 607 255 6249.
E-mail address: tdf1@cornell.edu (T.D. Fox).

0167-4889/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.05.004

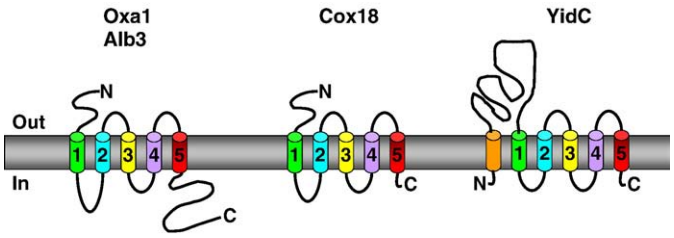

Fig. 1. Comparison of the general architectures of Oxa1-related proteins. The relative spacing of helices number 2–4 is largely conserved. A large internal C-terminal domain is present in mitochondrial Oxa1 and chloroplast Alb3, but absent in mitochondrial Cox18 and bacterial YidC. *E. coli* YidC has an N-terminal 'anchor' TM domain and long periplasmic N-terminal loop not found in the organellar proteins or related proteins from Gram positive bacteria. See text for references.

[13,16–21]. Oxa1 and Cox18 have an N_{out}–C_{in} orientation verified through protease protection experiments [13,16,17]. The hydrophilic domains of Oxa1 adhere to the positive inside rule [22] where the soluble N-terminus and loop between TM2 and TM3 bear net negative charges while the C-terminus and matrix localized loops bear net positive charges [13]. The conservation between species of this charge distribution, despite low sequence similarity, suggests its importance in topogenesis.

Mitochondrial inner membranes typically contain proteins from two distinct Oxa1 subfamilies (Fig. 2). Those proteins most closely related to *S. cerevisiae* Oxa1 have long (roughly 100 residues) hydrophilic tails at their C-terminal ends. Those most closely related to Cox18 of *S. cerevisiae* (termed Oxa2 in *Neurospora crassa*) lack long hydrophilic C-terminal domains [17]. While the amino acid sequence conservation between the Oxa1 and Cox18 subfamilies is weak, their homology is clear from both their TM core structure and functional complementation studies (described below). Divergence of the Cox18 subfamily of mitochondrial proteins from those closely resembling *S. cerevisiae* Oxa1 appears to have occurred prior to the divergence of animals, plants and fungi [17]. The Oxa1-related thylakoid membrane chloroplast protein, Alb3, appears to have an independent origin, presumably from *Cyanobacteria* [17].

The bacterial members of this family are known as YidC proteins, of which the *Escherichia coli* example is by far the best studied [21,23,24]. *E. coli* YidC, like those of other Gram negative bacteria, has five TM domains corresponding to those of Oxa1, and an additional anchor TM domain near the N-terminus: the protein has a large periplasmic loop between its first two TM domains, and N-in, C-in topology [15,25,26]. The large periplasmic domain linking the N-terminal anchor to the first TM domain of the conserved core domain has been crystallized [27–30]. This domain forms a twisted β-sandwich with an α-helical linker which orients the sandwich near the core TM domains. A portion of this periplasmic domain mediates an interaction with SecF [31], although this is not essential for inner-membrane biogenesis or cell viability [25]. Oxa1-like proteins of Gram positive bacteria (which lack an outer membrane) lack the additional anchor TM domain and large extra-cellular domain [21,32].

The presence of Oxa1-related proteins in several species of *Archaea* is strongly suggested by genome sequencing [6,11,12]. However, these proteins appear to lack two of the TM domains (numbered 3 and 4 in Fig. 1) found in the mitochondrial and bacterial proteins. The function of these proteins in *Archaea* has not been experimentally studied. Nevertheless, it appears that Oxa1-related proteins may have evolved before divergence of the three major domains of life [6,11,12].

To date, eukaryotic Oxa1-related proteins have been found in energy-transducing organelles that contain their own genetic systems, but no where else in the cell. Interestingly, genes for these proteins cannot be identified in the genome sequences of *Giardia lamblia* or *Trichonomas vaginalis*. These unicellular eukaryotic parasites contain double-membranous organelles related to mitochondria, but lack respiratory complexes and organelle genetic systems [33]. Thus, it is plausible to speculate that Oxa1-related proteins were lost in these lineages since they were no longer needed to insert organelle-encoded proteins into the inner membrane.

3. Regulation, synthesis and import of Oxa1 and Cox18 in various organisms

Both Oxa1 and Cox18 are nuclear-encoded, synthesized on cytoplasmic ribosomes and imported into mitochondria. Interestingly, *S. cerevisiae OXA1* belongs to a divergent transcription unit also controlling the expression of the *PET122* gene, coding a translational activator of the mitochondrial *COX3* mRNA [34]. The expression of the two nuclear genes *OXA1* and *COX18* clearly differs. The *OXA1* mRNA is abundant while the *COX18* mRNA is difficult to detect, both in yeasts and human [20,35]. Estimates of protein abundance based on accumulation of GFP fusion proteins are 6550 and 468 molecules per cell for Oxa1 and Cox18, respectively [36]. The synthetic respiratory haplo-insufficiency of *COX18/cox18* heterozygotes producing a Cox2 fusion protein inside mitochondria [16] probably reflects the low level of *COX18* expression.

Northern blot analysis has shown that the human gene for Oxa1, *OXA1L*, exhibits tissue specific expression [35]. However, the expression of yeast *OXA1* is not altered by either the carbon source or by respiratory deficiency [37,38]. In *Schizosaccharomyces pombe* the two *COX18* mRNAs are differentially expressed depending on the carbon source [35].

The 3′-UTRs of *OXA1* and *COX18* mRNAs of *S. cerevisiae* contain binding sites for Puf3, an RNA binding protein located at the cytosolic face of the mitochondrial outer membrane that regulates mitochondrial biogenesis [39,40]. The two mRNAs are clearly enriched in mitochondrion-bound polysomes suggesting that *in vivo* their import could be co-translational [41,42]. *In vitro* import experiments with isolated *S. cerevisiae* mitochondria and radio-labeled precursors indicate that the import of these two proteins requires the TOM/TIM import machinery, the membrane potential, the import motor mtHsp70, and matrix ATP [2]. This process converts the precursors to mature forms after cleavage of a long presequence, 42 aa in the case of *S. cerevisiae* Oxa1, by the mitochondrial processing peptidase [13,17,19,43,44]. Interestingly, after mature Oxa1 and Cox18 become located in the matrix following import into mitochondria isolated from an *Δoxa1* strain or a thermosensitive mutant (*oxa1-ts1402*), they cannot be inserted normally into the inner-membrane [17,45]. This lack of insertion could be due either to a direct role for Oxa1 in its own insertion (and that of Cox18p), or to the diminished membrane potential of the mutant mitochondria. In any event, a *Δoxa1* mutant strain can be restored to respiratory proficiency by the introduction of a plasmid carrying the *OXA1* gene, showing that at least some Oxa1 can be inserted into an inner-membrane previously devoid of Oxa1.

Oxa1 appears to form a homotetrameric complex as judged by blue-native PAGE and superose size exclusion chromatography [44,46,47]. Similarly, Cox18 migrates as a high molecular complex of unknown composition, but distinct from the Oxa1 complex [17]. Mutant forms of Oxa1 are highly sensitive to degradation by m-AAA proteases and the metallopeptidase Oma1 in *S. cerevisiae* [48].

4. Consequences of Oxa1 deficiency in various organisms

The *OXA1* gene was first isolated from *S. cerevisiae* by functional complementation of a respiratory deficient nonsense mutant in the W_{106} codon (*oxa1-79*, Kermorgant and Dujardin unpublished, [37]) and a $L_{240}S$ missense mutation in the loop between TM2 and TM3 (Fig. 3) (*oxa1-ts1402*, [49]). The *oxa1-79* strain lacked spectrally detectable cytochrome aa_3 and showed reduced splicing of both the *COX1* and *CYTb* precursor transcripts. Removal of all mitochondrial