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Review

Mitochondrial protein import machineries and lipids: A functional connection ☆

Natalia Gebert [a,b], Michael T. Ryan [c], Nikolaus Pfanner [a,b], Nils Wiedemann [a], Diana Stojanovski [c,*]

[a] Institut für Biochemie und Molekularbiologie, ZBMZ, Universität Freiburg, 79104 Freiburg, Germany
[b] Centre for Biological Signalling Studies BIOSS, Universität Freiburg, 79104 Freiburg, Germany
[c] La Trobe Institute for Molecular Sciences and Centre of Excellence for Coherent X-ray Science, La Trobe University, 3086 Melbourne, Australia

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ABSTRACT

Protein trafficking and translocation are essential processes in even the simplest living cells. The compartmentalisation within eukaryotic cells places a very high demand on the fidelity of protein trafficking and translocation, since a large percentage of the cell's protein complement is inserted into, or translocated across membranes. Indeed, most mitochondrial proteins are imported from the cytosol into the organelle and reach their final destination with the assistance of versatile translocation machineries. The first components involved in mitochondrial protein import were identified about 20 years ago and over the last two decades many new factors and machineries have been brought to light. However, in spite of these discoveries we still have much to explore regarding the molecular mechanisms that distinguish the different mitochondrial import pathways. In particular, an open question that requires deeper exploration is the role of lipids and lipid modifying enzymes in this process. Mitochondrial biogenesis requires the coordinated synthesis and import of both proteins and phospholipids, however, these have typically been considered as distinct research fields. Recent findings have placed phospholipids at the forefront of research dealing with mitochondrial biogenesis, in particular their role in the regulation of mitochondrial transport machineries. This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

Contents

Abbreviations: CDP-DAG, CDP-diacylglycerol; CL, cardiolipin; ER, endoplasmic reticulum; MDM, mitochondrial distribution and morphology; MIA, mitochondrial intermembrane space assembly; MLCL, monolysocardiolipin; Mmm1, maintenance of mitochondrial morphology; mtHsp70, mitochondrial heat shock protein 70; PA, phosphatidic acid; PAM, presequence translocase-associated motor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; PI, phosphatidylinositol; PS, phosphatidylserine; SAM, sorting and assembly machinery; Tam41, translocator assembly and maintenance protein 41; TIM22, carrier translocase of inner membrane; TIM23, presequence translocase of inner membrane; TOM, translocase of outer membrane

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* Corresponding author.
E-mail address: D.Stojanovski@latrobe.edu.au (D. Stojanovski).

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1. Introduction

Compartmentalisation of proteins within organelles of a eukaryotic cell serves to coordinate and separate metabolic processes. However, this poses a challenge for the cell, since it has to ensure that all nuclear-encoded and cytosolically synthesised proteins are delivered to the appropriate cellular compartment to carry out their function. The fact that mitochondria have their own genome does not liberate them from cellular control, as only a few proteins (8 and 13 polypeptides in yeast and humans, respectively) are encoded by the mitochondrial genome. Nuclear genes encode all remaining proteins,

approximately 1000 proteins in the baker's yeast *Saccharomyces cerevisiae* [1–3]. Therefore, mitochondrial biogenesis entails a coordinated effort between both genomes to ensure the simultaneous synthesis and assembly of its entire protein complement.

The presence of four mitochondrial subcompartments, the outer membrane, intermembrane space, inner membrane and matrix, can pose a challenge for successful sorting of nuclear encoded precursors, but the organelle has in place multiple machineries that operate in a collaborative manner to ensure the fidelity of this process. However, mitochondria also depend on the integration of new lipids for their biogenesis, some of which are synthesised in the organelle and others that need to be transported into the organelle from other cellular compartments. Given that phospholipids provide the environment for the assembly of translocation machineries, their influence on translocation events is likely to be significant. Indeed, recent studies have uncovered surfacing connections into the role of phospholipids on mitochondrial translocation machineries and ultimately protein import into the organelle. Herein, we will explore these two aspects. We will first describe the basic principles of the mitochondrial protein import machinery and then discuss the emerging link to phospholipids.

2. Mitochondrial protein import

The identification and characterisation of most mitochondrial translocation components have employed fungal models, in particular *S. cerevisiae*, however, the core machinery is conserved in plants and humans [1]. Indeed, multiple mitochondrial import pathways have now been well defined (Fig. 1), dramatically changing earlier perspectives that protein import into the organelle was mediated via one main pathway. These alternative import pathways adhere to some common features including: (1) specificity of substrates for precise cellular locations mediated by "organelle specific" targeting elements; (2) "receptors" on target membranes that serve as docking sites for incoming precursors; (3) translocation channels formed by membrane-embedded protein complexes; and (4) precursor translocation through narrow translocation pores typically in an unfolded or loosely folded conformation.

The vast majority of mitochondrial-destined proteins are synthesised on cytosolic ribosomes as precursor proteins. It is generally assumed that the majority of precursors are imported in a post-translational manner. However, a co-translational mode of protein translocation into mitochondria is likely for some precursors [4,5], though it is currently unknown if and which fraction of proteins are imported into mitochondria during synthesis on ribosomes and how tight the coupling between translation and translocation would be. Therefore, the typical journey of a mitochondrial precursor begins following its synthesis in the cytosol, where it must overcome the boundaries imposed by molecular crowding and find its way to the organelle. This efficient trafficking of precursors through the cytosol is promoted by both cytosolic factors [6] and organelle specific targeting elements that serve as "zip codes" to deliver the precursor to a corresponding receptor(s) on the mitochondrial surface. These zip codes also promote sorting of the precursor to the correct sub-mitochondrial location. Mitochondrial targeting elements exist as either individual or multiple units scattered along the length of the precursor and vary markedly in terms of sequence, structure and location, reflecting their role in alternative mitochondrial sorting routes. The "classical" mitochondrial targeting signal is a cleavable N-terminal positively charged sequence, termed a presequence, which directs proteins to the mitochondrial matrix, inner membrane and in a

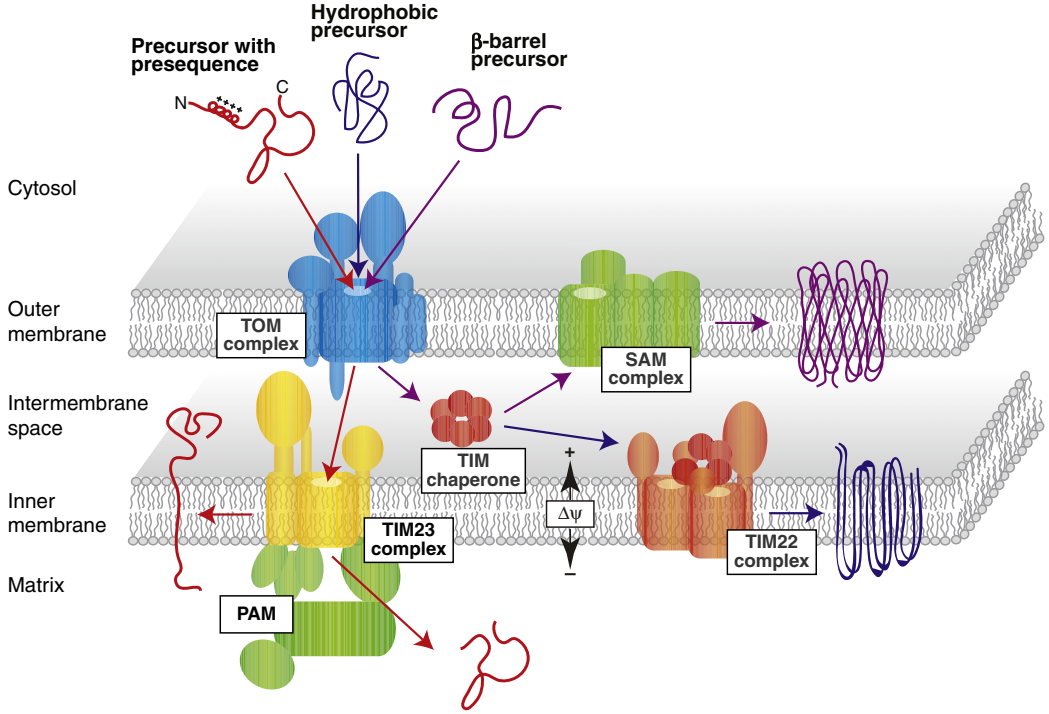

Fig. 1. The protein import machinery of the mitochondrial membranes. The greater part of all mitochondrial proteins is encoded by nuclear genes and are consequently synthesised as precursor proteins on cytosolic ribosomes. These undeveloped proteins must be targeted to and imported into mitochondria where they can acquire their functional and mature state. The translocase of the outer mitochondrial membrane (TOM complex) is the main entry gate into mitochondria and through a series of interactions with TOM complex receptors, precursors are guided to the "pore" of the complex in order to traverse the outer membrane. Upon outer membrane translocation different sorting pathways are initiated depending on the individual or multiple targeting elements contained within the precursor. Precursors possessing an N-terminal presequence are sorted to the presequence translocase of the inner membrane (TIM23 complex) in a membrane potential ($\Delta\psi$)-dependent manner. Complete translocation of precursors into the mitochondrial matrix is an ATP-driven process and requires the action of the presequence translocase-associated motor (PAM). Hydrophobic membrane proteins of the mitochondrial outer and inner membrane belonging to the β-barrel and carrier families, respectively, exploit the TIM chaperones of the intermembrane space for their passage through this aqueous environment. Following traffic through the intermembrane space, β-barrel precursors are directed to the sorting and assembly machinery (SAM complex) for outer membrane integration and carrier precursors are delivered to and inserted into the inner membrane by the $\Delta\psi$-driven carrier translocase (TIM22 complex).