Biochimica et Biophysica Acta 1793 (2009) 33–41

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Thirty years of protein translocation into mitochondria: Unexpectedly complex and still puzzling

Dejana Mokranjac, Walter Neupert *

Institute for Physiological Chemistry and Munich Center for Integrated Protein Science CIPS[M], Ludwig-Maximilians University, Butenandtstr. 5, 81377 Munich, Germany

ARTICLE INFO

Article history:
Received 3 June 2008
Received in revised form 16 June 2008
Accepted 26 June 2008
Available online 12 July 2008

Keywords:
Mitochondria
Protein translocases
Protein transport
Protein sorting

ABSTRACT

Mitochondria are essential organelles of the eukaryotic cells that are made by expansion and division of pre-existing mitochondria. The majority of their protein constituents are synthesized in the cytosol. They are transported into and put together within the organelle. This complex process is facilitated by several protein translocases. Here we summarize current knowledge on these sophisticated molecular machines that mediate recognition, transport across membranes and intramitochondrial sorting of many hundreds of mitochondrial proteins.

1. Introduction

Mitochondria are organelles present in virtually all eukaryotic cells. They function on the crossroads of life and cell death. They produce ATP as the energy source for the cell and house a number of essential biosynthetic pathways but, on the other hand, are key triggers of apoptosis [1–3]. Mitochondria are made up of two membranes, the outer and the inner mitochondrial membrane, which delimit two aqueous subcompartments, the intermembrane space (IMS) and the innermost mitochondrial matrix. They are composed of a large number of components belonging to different classes: proteins, nucleic acids, lipids and solutes. These components are synthesized in different parts of the cell and put together in the mitochondria by a number of supramolecular machines.

During the past 30 years, a major branch of research on the biogenesis of mitochondria has focused on transport and sorting of proteins, although a wealth of information has also been obtained from studies on the synthesis and metabolism of DNA and RNA as well as on biosynthesis, intracellular transport and turnover of the many lipid species of the mitochondrial membranes [4–7]. It is estimated that, depending on the organism, mitochondria contain about 1000–2000 different proteins. They are encoded in two different genomes in the cell. The genes for roughly 99% of mitochondrial proteins are present in the nuclear DNA and for only a very few of them in the mitochondrial DNA (mtDNA). Proteins encoded by the nuclear DNA are synthesized in the cytosol in the form of preproteins and are transported into mitochondria in a preferentially posttranslational manner.

Mitochondrial preproteins carry targeting signals which are recognized by cytosol-exposed receptors present in the mitochondrial outer membrane. After initial recognition by the receptors, targeting signals are deciphered by mitochondrial translocases, complex molecular machines responsible for intramitochondrial sorting of preproteins. In the last 20 years the number of mitochondrial translocases identified and their components has been steadily growing. Furthermore, during more recent years it became obvious that transport of proteins into mitochondria is closely connected to their dynamics and structure, as well as to apoptosis and signaling [8–10]. This article will summarize current knowledge of protein transport into and within mitochondria.

2. Mitochondrial targeting signals

Proteins are targeted to mitochondria by the virtue of specific, mitochondrial targeting signals (Fig. 1) [11–14]. As we are completing the mitochondrial proteome it is becoming obvious that these signals are very diverse in nature and can be present anywhere in the polypeptide sequence. Roughly half of mitochondrial proteins are synthesized with an N-terminal extension called a matrix targeting sequence (MTS) or presequence. MTSs are not conserved in their primary sequence but they all share the propensity to form an amphipathic helix with one positively charged and one hydrophobic surface. They are necessary and sufficient to target a passenger protein into the mitochondrial matrix. Once in the matrix, MTSs are proteolytically removed by the matrix processing peptidase. MTS is normally present at the N-terminus of preprotein though at least one example exists in which the presequence is found on the C-terminus, the mitochondrial DNA helicase, Hmi1 [15]. The other half of mitochondrial preproteins contain noncleavable, mostly internal targeting signals. In the

* Corresponding author. Fax: +49 89 2180 77093.
E-mail address: Neupert@med.uni-muenchen.de (W. Neupert).

0167-4889/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.06.021

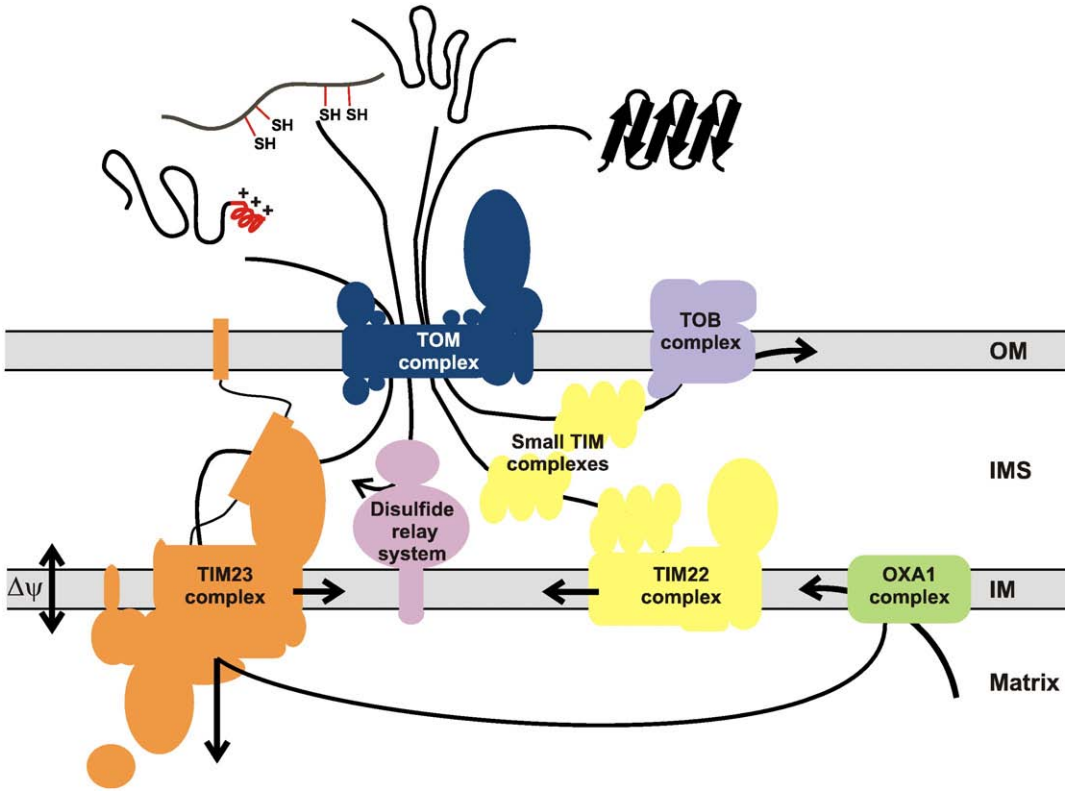

Fig. 1. Overview of pathways of translocation and sorting of proteins into mitochondria. See text for details. OM, outer mitochondrial membrane; IMS, intermembrane space; IM, inner mitochondrial membrane.

majority of cases they are still poorly characterized. Several types of internal targeting signals apparently exist that can target proteins to the outer and inner mitochondrial membranes and to the intermembrane space. At least three types of proteins are found in the outer mitochondrial membrane [16]. Signal-anchored and tail-anchored proteins are integrated into the membrane with a single α-helical transmembrane domain close to the N- or the C-terminus of the protein, respectively. In contrast, β-barrel proteins cross the outer membrane several times with a number of β-strands. Moderate hydrophobicity of the transmembrane domain and positive charges flanking this region appear to be important for targeting of signal-anchored proteins to the mitochondrial outer membrane [17]. Insertion of tail-anchored proteins likely requires a net positive charge located C-terminally to the tail-anchor domain [18]. In contrast, β-barrel proteins cross the outer membrane several times with a number of β-strands. Recently a signal was identified which is necessary for integration of β-barrel precursors into the outer membrane [19]. It is present in the C-terminal region of β-barrel proteins and consists of a large polar residue, an invariant glycine and two large hydrophobic residues. Still, the determinants in the precursor forms of β-barrel proteins that enable them to cross the outer membrane through the TOM complex remain enigmatic. A group of intermembrane space proteins contain conserved cysteine residues which are not only important for their function but also for their translocation into mitochondria [20]. Hydrophobic inner membrane proteins of the carrier family contain poorly defined internal targeting signals which appear to be composed by both transmembrane segments and connecting loops [21]. A number of known mitochondrial proteins seem not to contain any of these signals suggesting that the repertoire is even more diverse. Different targeting signals are recognized by different protein translocases and different proteins are thereby sorted into different mitochondrial subcompartments.

3. Overview of mitochondrial translocation pathways

The TOM (translocase of the outer membrane) complex is the major translocase of the outer membrane. It is used by all mitochondrial proteins analyzed so far for transport across the outer membrane [11–14]. Depending on the type of targeting signal, the TOM complex cooperates with other mitochondrial translocases to sort proteins into the outer membrane, the intermembrane space, the inner membrane and the matrix. Transport of MTS-containing preproteins requires the concerted action of the TOM complex and the TIM23 complex in the inner membrane. Cooperation of TOM, small TIM and TIM22 complexes leads to insertion of hydrophobic membrane proteins of the carrier family into the inner membrane. Import of cysteine-containing proteins in the intermembrane space is dependent on the combined action of the TOM complex and the Mia40-Erv1 disulfide relay system in the intermembrane space. Transport and insertion of β-barrel proteins into the outer membrane involves the TOM complex, the small TIM complex in the intermembrane space and the TOB/SAM complex in the outer membrane. Signal-anchor proteins in the outer membrane apparently require only the receptors of the TOM complex but not the translocation pore [22], whereas tail-anchor proteins seem to insert into the outer membrane in a TOM-independent manner [23,24]. A number of inner membrane proteins are synthesized with a cleavable presequence. Some of them contain an additional stop-transfer signal which leads to translocation arrest at the level of the TIM23 complex and their insertion into the inner membrane. The others are, however, first completely transported into the matrix and only then inserted into the inner membrane with the help of Oxa1 complex. This process resembles protein insertion into the bacterial inner membrane and it is therefore termed conservative sorting. The Oxa1 complex is also involved in insertion into the inner membrane of proteins encoded by the mtDNA and translated on mitochondrial ribosomes tethered to the internal face of the inner membrane.