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Review

Understanding the molecular mechanism of protein translocation across the mitochondrial inner membrane: Still a long way to go☆

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ABSTRACT

In order to reach the final place of their function, approximately half of the proteins in any eukaryotic cell have to be transported across or into one of the membranes in the cell. In this article, we present an overview of our current knowledge concerning the structural properties of the TIM23 complex and their relationship with the molecular mechanism of protein transport across the mitochondrial inner membrane. This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

Contents

1. Introduction

The vast majority of proteins in any eukaryotic cell are synthesized on cytosolic ribosomes. However, only about half of these proteins function in the cytosol; the rest function in one of the cellular organelles or in the plasma membrane. In order to reach their final destination, they must be translocated across or into one of the various membranes in the cell. Intracellular sorting of proteins relies on the presence of a specific targeting signal on the one hand and on complicated proteinaceous machineries called protein translocases on the other. Translocases recognize the targeting signals, and subsequently mediate the transport of proteins encompassing those signals across or into the specific organellar membranes [1,2].

☆ This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

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Mitochondria contain a double layer of membranes, the outer and inner mitochondrial membranes, which define two aqueous compartments, the intermembrane space (IMS) and the innermost mitochondrial matrix. The transport of proteins into mitochondria is a particularly demanding task as it not only requires targeting to the organelle but it also necessitates the proper sorting of the proteins to the correct intramitochondrial compartment. With the exception of a few proteins encoded by the mitochondrial DNA, all mitochondrial proteins are synthesized in the cytosol in the form of precursor proteins [3–6]. Mitochondrial proteins carry a large number of different targeting signals. However, they are all recognized at the mitochondrial surface by receptors of the TOM (translocase of the outer mitochondrial membrane) complex. The TOM complex, covered in more detail in another article of this special issue (Rapaport D) is composed of the receptor proteins Tom20, Tom70 and Tom22, the ß-barrel protein Tom40 which forms a translocation channel, and three small Tom proteins, Tom5, Tom6 and Tom7, which most likely contribute to the dynamics of the TOM complex but may also have a more direct role in translocation of proteins (Fig. 1). Essentially all mitochondrial proteins are transported across the outer membrane through the translocation channel of the TOM complex. However, the various mitochondrial import pathways diverge at the intermembrane space (IMS) side of the TOM complex. So far, three proteins, Tom40, Tom22 and Tom7, have been implicated in the formation of this so called trans binding site of the TOM complex. It is likely that the trans site has binding sites for translocation machineries present in the IMS and/or inner membrane so that proteins are more efficiently transported into the organelle. The various import pathways present in mitochondria are thoroughly covered in several articles in this special issue. Here we will concentrate on the translocation of proteins across the mitochondrial inner membrane mediated by the TIM23 complex.

2. The translocase of the inner membrane of mitochondria — the TIM23 complex

Essentially all matrix proteins and a large number of inner membrane proteins are targeted to mitochondria by a positively charged N-terminal segment called a presequence or matrix targeting signal (MTS). The transport of these precursor proteins into the organelle depends on the cooperative action of the TOM complex in the outer membrane and the TIM23 complex in the inner membrane. Using the energy of the membrane potential across the mitochondrial inner membrane and ATP in the matrix, the TIM23 complex mediates translocation of proteins across and their insertion into the mitochondrial inner membrane. The large number of components involved, the diversity of tasks it performs and the energy sources it requires, make the TIM23 complex the most complicated of all mitochondrial translocases. The precursor proteins are recognized at the outlet of the TOM channel by the IMS-exposed receptor proteins Tim50 and Tim23, which guide them to the translocation channel formed by Tim23 and, possibly, Tim17 [7–13]. This part of the complex is sufficient for membrane-potential dependent transport of the presequence into the matrix. However, the translocation of the complete polypeptide chain into the matrix requires the ATP-dependent action of the import motor of the TIM23 complex [14]. The ATP-consuming subunit of the complex is mtHsp70. It binds and releases segments of the translocating chain in an ATP hydrolysis regulated manner [15–17]. This process is regulated by the various co-chaperones of mtHsp70: Tim44 which recruits mtHsp70 to the translocation channel in the inner membrane [18–22], Tim14 (Pam18) which stimulates ATP hydrolysis by mtHsp70 [23–25], Tim16(Pam16) which controls the activity of Tim14 [26–28] and Mge1 which stimulates the release of ADP [29–31]. Repeated cycles of binding to and release from mtHsp70 lead to the vectorial transport of the polypeptide chain into the matrix [32]. This pathway is followed by all precursor proteins in which the N-terminal presequence is their only targeting signal. However, if the translocating precursor protein contains an additional signal which is recognized by the TIM23 complex as the lateral sorting signal [33], the complex undergoes a conformational change which leads to the lateral opening of the translocation channel and insertion of the precursor protein into the inner membrane [10,34]. Thus, the TIM23 complex is actively remodeled during sorting of proteins into two different compartments of mitochondria. The subunits of the TIM23 complex involved in recognition of the lateral sorting signal are largely unclear as are the molecular mechanisms underlying the lateral opening of the TIM23 complex and subsequent insertion of the membrane spanning

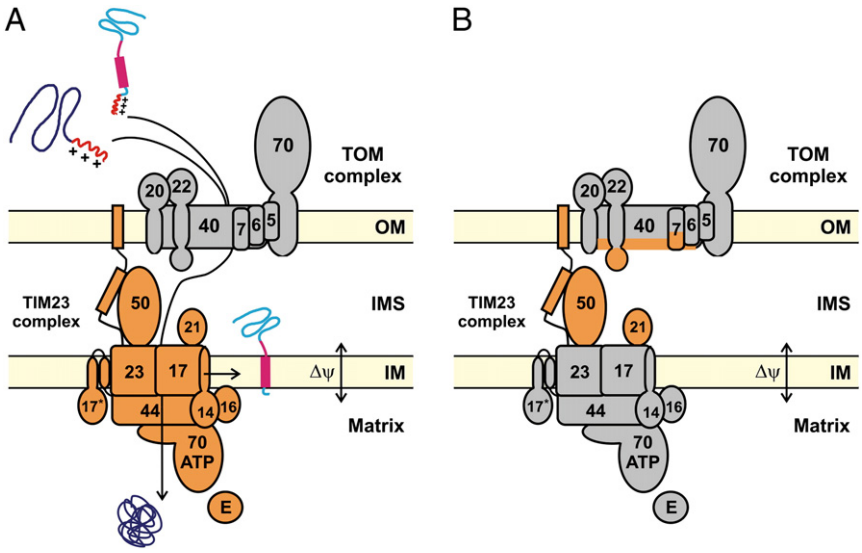

Fig. 1. Import pathway of precursor proteins with N-terminal presequences. (A) Import of precursor proteins with N-terminal presequences (in red) requires a cooperative action of the TOM complex (in grey) in the outer membrane and the TIM23 complex (in orange) in the inner membrane. If the presequence is the only targeting signal present, the precursor protein will be translocated completely into the matrix. If, however, an additional lateral sorting signal (in magenta) is present, it will be recognized by the TIM23 complex and the precursor protein will be laterally released into the inner membrane. (B) Several components of the TOM and TIM23 complexes were implicated to contribute to the cooperation of the complexes in the intermembrane space. They are indicated in orange, and the rest are shown in grey. OM, outer mitochondrial membrane; IMS, intermembrane space; IM, inner mitochondrial membrane; numbers indicated the molecular weights of the respective TOM and TIM components. 17* indicates Pam17.