Moving proteins from the cytosol into mitochondria

A. Chacinska and P. Rehling
Institut für Biochemie und Molekularbiologie, Universität Freiburg, Hermann-Herder-Strasse 7, D-79104 Freiburg, Germany

Abstract
Mitochondria of the yeast Saccharomyces cerevisiae contain at least 750 different proteins, which perform diverse roles. Most of these proteins (approx. 99%) are translated on cytosolic ribosomes, and their import into mitochondria is essential for mitochondrial function. Proteinaceous machineries of great complexity, the so-called translocases, in the mitochondrial membranes mediate the import of these proteins.

Of the mitochondrial proteome, only eight stable proteins are encoded and translated in mitochondria [1,2]. Therefore mitochondria depend on a constant influx of proteins from the cytosol into the organelle to fulfil their cellular tasks. Preproteins destined for the mitochondria are recognized and sorted to their functional location by the translocases of the outer and inner mitochondrial membranes [3–6].

Mitochondrial precursor proteins contain specific targeting signals within their primary sequence that are initially recognized by specialized receptor proteins with cytosolic domains residing in the outer mitochondrial membrane. The receptors associate with the GIP (general import pore) of the outer membrane, a 450-kDa membrane protein complex that contains the Tom40 protein as its central channel-forming unit. Receptors and GIP together form the TOM (translocase of the outer mitochondrial membrane) complex, which serves as the general mitochondrial entry gate for the unfolded precursor proteins. Proteins that are destined for the outer membrane or an inner subcompartment are transported through the Tom40 pore [3–6]. Recent analyses have shown that the TOM complex is involved in the insertion of proteins with single-transmembrane segments into the outer membrane. Proteins that have a more complex topology, such as outer-membrane β-barrel proteins, cannot be inserted into the membrane by the TOM complex alone. Membrane insertion of β-barrel proteins apparently requires an additional translocation machinery in the outer membrane, termed the SAM (sorting and assembly machinery of the outer membrane) complex (Figure 1). The SAM complex receives β-barrel proteins from the TOM complex and mediates their membrane insertion by an as yet unknown mechanism [4–8]. It is interesting to note that the central component of the SAM complex (Sam50) is evolutionarily conserved and that a homologue participates in inserting bacterial outer-membrane β-barrel proteins. Therefore it can be assumed that the mechanism of insertion is similarly conserved.

Proteins of the inner membrane also need to be translocated via the TOM complex. This group includes members of the large group of metabolite carriers that mediate the vital transport of small molecules across the inner membrane (Figure 1). These proteins contain multiple transmembrane segments. Owing to their hydrophobic nature, these precursors interact with different chaperones on their trip to the inner membrane. After synthesis of the precursor in the cytosol, chaperones of the Hsp70 (heat-shock protein 70) class (and Hsp90 in mammals) protect the precursor molecule against aggregation and assist it in recognition at the mitochondrial surface. After dissociation from the receptors, the precursor is transported across the outer membrane. As soon as hydrophobic segments of the carrier precursor become exposed to the intermembrane space, the Tim9–Tim10 complex binds to it, prevents backsliding and protects the precursor against the aqueous surrounding. At the inner membrane, a specialized translocation machinery, the twin-pore carrier translocase (also known as the TIM22 complex) receives the precursor from the Tim9–Tim10 complex and catalyzes its insertion into the inner membrane. This process uses the membrane potential across the inner membrane as an external energy source [4–6].

Outer-membrane proteins and carrier proteins use targeting signals that are not well defined; what is known instead is that they are internal signals that may be distributed over the length of the protein. In contrast, proteins destined for the mitochondrial matrix use N-terminal signals, the so-called presequences, as a means of targeting to mitochondria (Figure 1). These are positively charged and cleaved off in the matrix after import. In addition, some precursor proteins destined for the inner membrane or the intermembrane space also possess presequences. Specific receptors of the TOM complex recognize the presequence of a precursor protein at the surface of mitochondria and direct it into the GIP.
complex, which then facilitates outer-membrane translocation. Translocation of matrix-destined precursor proteins across the intermembrane space and the inner membrane depend on a dedicated inner-membrane translocation machinery specific for this class of precursors – the presequence translocase – that tightly co-operates with the TOM complex [3–6]. This multi-protein complex is composed of two modules, the membrane-embedded TIM23 complex and the PAM (presequence translocase-associated import motor) complex, which binds to it from the matrix side of the inner membrane [9–12] (Figure 2). The presequence translocase consists of seven protein components, all of which are essential for cell viability. The inner membrane proteins Tim17, Tim23 and Tim50 form the TIM23 complex. Tim50 acts as the first of these proteins in precursor transport since it contacts the precursor as soon as it emerges from the TOM complex. Tim23 exposes a domain of approx. 100 amino acids into the intermembrane space that has been suggested to serve as a receptor for presequences at the inner membrane. In addition, Tim23 forms the pore of the translocase through which precursors pass the inner membrane. A physical interaction of the intermembrane space portion of Tim50 with the receptor domain of Tim23 helps to direct the precursor to the pore. Translocation across the inner membrane requires the membrane potential $\Delta \psi$ as an initial driving force. The $\Delta \psi$ activates the Tim23 channel and drives the presequence across the inner membrane electrophoretically [3–6]. The TIM23 complex alone is capable of inserting certain presequence proteins into the inner membrane. These proteins usually possess a hydrophobic sorting signal adjacent to the presequence, which directs them into the inner membrane. In contrast, proteins that need to be completely translocated into the matrix depend on a second driving force for translocation, which is provided by the PAM complex (Figure 2). The central PAM component is the mtHsp70 (mitochondrial Hsp70) that binds to the incoming unfolded precursor. Several ATP-dependent cycles of successive binding of mtHsp70 to the precursor provide...
an inward-directed driving force that results in the complete translocation of the precursor [3–6]. This cycling of mtHsp70 at the presequence translocase has to be tightly regulated and co-ordinated with the TIM23 complex; therefore, three membrane proteins co-operate with mtHsp70 in protein transport. Tim44 has no direct effect on the ATPase activity of mtHsp70, but seems to be involved in loading mtHsp70 on to precursor proteins in transit. Binding of mtHsp70 to precursor proteins requires that the bound ATP be hydrolysed. The ATPase activity of mtHsp70 is stimulated by an interaction with the co-chaperone Pam18, an integral membrane protein that contains a DnaJ-like domain exposed to the matrix [9–11]. In addition, Pam16 has recently been found as a novel PAM constituent. Although Pam16 displays sequence similarity to DnaJ proteins, it is not capable of stimulating mtHsp70 [12,13]. However, Pam16 interacts with the J-domain of Pam18 and the two proteins form a stable complex. Pam16 was found to be necessary for the association of Pam18 with the presequence translocase [12,13]. A hypothetical but interesting model as to the function of Pam16 is that it might regulate the activity of Pam18 at the channel. Eventually, the Mge1 protein mediates ADP/ATP exchange for mtHsp70 and, thus, primes it for the next round of precursor interaction.

In summary, recent years have brought new components of the translocation machineries and even new protein complexes that are involved in protein transport, which was unexpected. It appears that the processes underlying protein transport into mitochondria are much more complex and dynamic than we first anticipated and that mitochondria are still up for surprises.

We thank Dr R. Taylor and Dr N. Wiedemann for critical comments on this paper. Work in our laboratory is supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 388. A.C. was supported by an FEBS fellowship.

References

Received 7 July 2004