Ups delivery to the intermembrane space of mitochondria: a novel affinity-driven protein import pathway

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Mitochondria are comprised of two aqueous subcompartments: the matrix and the intermembrane space (IMS). Following their synthesis on cytosolic ribosomes, proteins destined to the matrix are threaded by N-terminal matrix-targeting sequences through TOM and TIM translocases in the outer and the inner membrane of the organelle. Proteins of the IMS often lack such presequences, and the principles mediating their uptake into mitochondria are diverse and poorly understood. In this issue, Potting *et al* (2010) and Tamura *et al* (2010) show that Ups proteins, a group of soluble IMS proteins critical for lipid homeostasis, adsorb to the IMS factor Mdm35 after their translocation across the outer membrane. Mdm35 thereby apparently functions as an intramitochondrial acceptor protein. The existence of affinity-driven import routes has been proposed before, but Mdm35 represents the first intramitochondrial acceptor protein that is identified.

About 50 different proteins were localized to the IMS so far and the list is steadily growing. Many of these components shuttle metabolites, proteins, metal ions or lipids between

both mitochondrial membranes. Ups proteins were recently identified in yeast as IMS proteins critical for the lipid composition of the inner membrane and, as a consequence, for the biogenesis of some inner membrane proteins and for mitochondrial morphogenesis (Sesaki *et al*, 2006). Baker's yeast contains three members of this family: Ups1, Ups2 and Ups3. They comprise a conserved protein domain, which is referred to as protein of relevant evolutionary and lymphoid interest (PRELI) domain. The human genome codes for at least four proteins with PRELI domains. Yeast mutants lacking Ups1 contain reduced levels of the mitochondria-specific lipid cardiolipin; in contrast, Ups2-deficient mitochondria contain even increased levels of cardiolipin, but diminished amounts of phosphatidylethanolamine (Osman *et al*, 2009; Tamura *et al*, 2009). Both cardiolipin and phosphatidylethanolamine are synthesized in mitochondria. Ups1 and Ups2 apparently have an antagonistic function in the regulation of lipid homeostasis. But it is unclear whether they influence the transport of lipid molecules to the mitochondrial inner membrane, the synthesis of lipids in mitochondria or the breakdown of lipids.

Figure 1 Mdm35 is a 'twin Cx₉C' protein that is imported into the IMS by the mitochondrial disulphide relay system. Mia40 and Erv1 facilitate the oxidation and folding of Mdm35 and thereby trap the protein in the IMS. Ups1 and Ups2 are recognized by the import receptors Tom20 and Tom22 and subsequently associate with Mdm35 and potential further factors (marked with '?') in mitochondria. These complexes regulate the lipid composition of the inner membrane. In the absence of Mdm35, Ups proteins do not efficiently accumulate in mitochondria because of their diminished import and increased degradation.

Whereas all matrix proteins use one common import pathway, IMS proteins embark on several import routes that differ in their principles and components (Herrmann and Hell, 2005; Chacinska *et al*, 2009; Endo and Yamano, 2009). (1) Some IMS proteins are synthesized with N-terminal-targeting signals, so-called bipartite presequences. They arrest the precursor at the level of the inner membrane and release the mature proteins upon cleavage of maturation proteases. (2) A second group of IMS proteins lacks presequences, but contains characteristic patterns of cysteine residues. Cysteine oxidation by the oxidoreductase Mia40 and the sulfhydryl oxidase Erv1 drives the import process. Substrates of this system form helix-loop-helix structures in which the two helices are connected by two parallel disulphide bonds. As the disulphide bonds are separated by three or nine residues, these proteins are named 'twin Cx₃C' and 'twin Cx₉C' proteins. The 'twin Cx₃C' proteins, also called small Tim proteins, serve as chaperones that usher hydrophobic inner membrane proteins from the TOM translocase to the inner membrane. The function of 'twin Cx₉C' proteins is less understood. Mitochondria contain 13 of these proteins that are conserved from fungi to animals (Gabriel *et al*, 2007; Longen *et al*, 2009). (3) A third group of proteins lacks both presequences and conserved cysteine residues. It was suggested that the association with specific binding partners in the IMS might drive their import process, but corresponding intramitochondrial acceptor proteins could not be identified thus far (Diekert *et al*, 1999).

Two studies published in this issue identified the 'twin Cx₉C' protein Mdm35 as a central component in the import of Ups proteins (Potting *et al*, 2010; Tamura *et al*, 2010). Mdm35, which is present in the IMS in much larger amounts than Ups proteins, functions as intramitochondrial complex partner of Ups1 and Ups2. Apparently, newly imported Ups proteins use Mdm35 as intramitochondrial docking site. Hence, Ups proteins may reach the IMS by sequential interactions with the outer membrane receptors Tom20 and Tom22 on the mitochondria surface and Mdm35 (Figure 1). It remains unclear whether Mdm35 interacts with Ups proteins during or after

their translocation. In the first case, the binding might be directly used to thread Ups proteins through the protein-conducting channel of the TOM complex. It should be noted that, although Mdm35 improves Ups import, it is neither essential nor rate limiting for translocation. It will be interesting to assess whether other 'twin Cx₉C' proteins exhibit similar functions as acceptor proteins or stabilizing factors.

In contrast to the receptors of the TOM complex, Mdm35 does interact permanently rather than transiently with Ups proteins. Hence, Mdm35 and Ups proteins form stable complexes in the IMS, which at least in the case of Ups2 presumably contain further, so-far unidentified factors. Whereas Potting *et al* (2010) and Tamura *et al* (2010) come to the same conclusion on the relevance of Mdm35 for Ups import, both studies disagree on the function of Mdm35 for already imported Ups proteins. Potting *et al* (2010) show that in the absence of Mdm35, imported Ups1 and Ups2 are rapidly degraded. Two IMS-oriented proteases of the mitochondrial quality control system, i-AAA (Yme1) and Atp23, cooperate in this process (Figure 1, right panel). Tamura *et al* (2010) do not observe this degradation and claim that Ups proteins stably accumulate in Mdm35-deficient mitochondria, albeit at severely reduced levels. It will be exciting to explore the potential function of the mitochondrial quality control system in the import of Ups proteins into the IMS and, thereby, in the regulation of the lipid composition of the inner membrane. A recent study reporting altered levels of phosphatidylethanolamine in i-AAA-deficient mitochondria already points to this direction (Nebauer *et al*, 2007).

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Conflict of interest

The author declares that he has no conflict of interest.

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