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Review

Delivery of nascent polypeptides to the mitochondrial surface

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Abstract

Thousands of polypeptides with diverse biochemical properties, some of which are extremely hydrophobic, are targeted from cytoplasmic ribosomes to the surface of mitochondria. Localised synthesis, as well as transient interactions with a wide array of molecular chaperones and other cytoplasmic factors, can promote productive interaction of mitochondrial proteins with the TOM complex to initiate protein import into mitochondria.

Keywords: Ribosome; polypeptide synthesis; mRNA localisation; Molecular chaperone; Protein import; Mitochondrion

It is reasonable to estimate that 10–20% of the genome from any given fungus, plant or animal encodes proteins that will be targeted to mitochondria. The transcripts from these genes are translated on cytoplasmic ribosomes with the newly synthesised precursor having all the targeting information encoded in the polypeptide, often in the form of N-terminal helix [1,2]. The nascent polypeptides must find their way to the organelle surface in order to engage with the TOM translocase and enter the mitochondrial sub-compartment (reference to TOM reviews in this edition).

A common feature of all mitochondrial precursor proteins is that they are more hydrophobic than other cytoplasmic proteins [3]. How do these unfolded, hydrophobic precursor polypeptides avoid aggregation? Also for precursor proteins to be translocated across the mitochondrial membranes in an unfolded state [4–6], two processes are at work in vivo to minimise aggregation and misfolding of mitochondrial precursor proteins: coupling of translation to translocation, and formation of transient, stabilising complexes with molecular chaperones and other cytoplasmic factors.

1. To what degree can translation and import be coupled for mitochondrial precursor proteins?

Unlike protein targeting to the endoplasmic reticulum [7,8], there is no known mechanism for tightly coupling translation of mitochondrial precursor proteins to their translocation into the organelle. While it is clear that translationally active ribosomes are present on the surface of mitochondria [9–11], it seems likely that the relative kinetics of translation and translocation determines the enrichment of polysomes encoding mitochondrial precursors on the organelle surface. Sequences within the mRNA might act as binding sites for factors that can transport mRNA to the vicinity of the mitochondria, or simply stabilise the mRNA to increase the number of rounds of translation each mRNA molecule can support [47]. The net effect will be the same: sufficient polysomes will find their way to the mitochondrial surface so that many precursor molecules will be translated directly into the TOM complex without being exposed to the cytosol, and would therefore have no chance to aggregate.

Fumarase, an enzyme of the Krebs cycle, is distributed between the cytosol and the mitochondrial matrix in yeast and provides an interesting case in point. A single transcript from the FUM1 gene is translated from a single ATG codon to yield a precursor form of fumarase with a targeting sequence at its amino terminus [12]. If the protein is fully translated, it will fold in the cytosol before being targeted to mitochondria. Upon import, the amino-terminus will enter the matrix for processing, but because this protein is not unfolded to allow complete passage through the mitochondrial membranes, this "posttranslational import" results in a processed protein that slides back out into the cytosol [13]. If ribosomes translating fumarase have docked with mitochondria, all subsequent fumarase precursors are fed into the

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mitochondria co-translationally and able to enter the matrix to fold into an active mitochondrial enzyme.

In a few cases, the mRNA encoding a mitochondrial precursor protein contains *cis* sequences to target the message to mitochondria so that the ribosomes engaged with these messages will only translate nascent precursor proteins in the vicinity of the organelle [48]. In situ hybridization of nucleic acid probes demonstrates clusters of polysomes synthesizing the $F_1\beta$ subunit of the F_1F_0-ATPase in halos around the mitochondria of rat hepatocytes [14,15]. Similarly, the message encoding the Atm1 transporter of *Saccharomyces cerevisiae* is targeted to the surface of mitochondria as a result of discrete RNA sequences, including one in the 3′-UTR [16]. However, in considering the total population of precursor molecules, it is clear that many are not targeted to mitochondria co-translationally. While the polysomes synthesizing the β-subunit of the F_1F_0-ATPase cluster around the mitochondria, polysomes synthesizing the α-subunit of the F_1F_0-ATPase are uniformly distributed throughout the cytoplasm [14]. While the *ATM1* mRNA is normally targeted to the surface of mitochondria, deletion of the 3′-UTR targeting signal does not prevent import of the Atm1 protein into mitochondria [16].

Since there is no absolute coupling of translation and translocation of mitochondrial precursor proteins, a large population of molecules of any given precursor will be translated on ribosomes distant from the organelle surface and be prone to folding and aggregation in the cytosol. However, several molecular chaperones and other factors are known to recognise and bind these unfolded, hydrophobic precursors and increase their probability of encountering the mitochondrial surface.

2. Molecular chaperones assist posttranslational translocation into mitochondria

2.1. Hsp70

The 70-kDa heat-shock protein (Hsc70) found in the cytosol of mammalian cells can assist mitochondrial precursors to achieve ‘‘import-competence’’ [17,18]. Immunodepletion of Hsc70 from reticulocyte lysates renders the precursor to ornithine transcarbamylase incapable of being imported, probably through misfolding and aggregation. Restoration of import-competence cannot be achieved by addition of Hsc70 after synthesis is complete, only during synthesis. This suggests that Hsc70 is acting on the precursor co-translationally to prevent it from aggregating or misfolding. Hsc70 has been shown to interact generally with nascent chains, not just with those representing mitochondrial precursor proteins [19,20].

In yeast, seven isoforms of Hsc70 are found in the cytosol [21]. One of these, Ssb1/2p, has been shown to be associated with ribosome and can be cross-linked to nascent chains [22,23], while another ribosome-associated Hsp70, Ssz1p, can promote import of ribosome-bound mitochondrial protein precursor [24]. Shutting down the expression of another isoform of Hsc70, Ssa1/2p, leads to accumulation of mitochondrial precursor proteins [25] and the purified Ssa1/2p stimulates the import of precursors into isolated mitochondria [26].

2.2. Hsp40

Hsp40 family members might act in partnership with specific Hsp70 partners to prevent aggregation and aid in the folding process. The Hsp40 Ydj1p is a partner to the Hsp70 Ssa1/2p [27,28] and mutants lacking fully functional Ydj1p have defects in their ability to import precursors into the mitochondria and the ER [27,29,30]. Ydj1 has a farnesyl moiety attached to its C terminus, allowing the protein, and perhaps its Hsc70 partners, to be localised to intracellular membranes [30]. A mutation in C terminus of Ydj1p that lacks the farnesyl moiety also accumulates mitochondrial precursor proteins in the cytosol [30].

2.3. Nascent-associated polypeptide complex (NAC)

The NAC is a heterodimeric protein that associates with ribosomes, and NAC can be cross-linked to various nascent chains including mitochondrial precursor proteins [31]. Yeast mutants lacking functional NAC display a slow growth phenotype on nonfermentable carbon sources and have defects in the accumulation of model proteins in the mitochondria, suggesting a role in mitochondrial biogenesis [32]. Purified NAC is sufficient to stimulate effective import of a nascent-chain of malate dehydrogenase into isolated mitochondria [33].

2.4. Ribosome-associated complex (RAC)

Another Hsp70–Hsp40 pair was identified recently, using ribosomes isolated from cells lacking NAC; a second factor was purified that can also stimulate import of nascent chains into purified mitochondria. The RAC is a heterodimeric complex consisting of Ssz1p and Zuo1p [24]. Both RAC and NAC can bind ribosome-nascent chain complexes in the absence of organelles, and probably assist the precursor to maintain a conformation that is compatible with binding to the TOM complex on the mitochondrial surface. While normally involved in the process, neither factor is essential since yeast strains lacking either factor can maintain normal mitochondrial function.

2.5. Mitochondrial import stimulation factor (MSF)

When the precursor form of adrenodoxin is synthesised in wheat germ lysates, it aggregates and cannot be imported by mitochondria. Addition of rat liver cytosol to such in vitro assays provides two factors that can stimulate import:

Hsc70 and MSF. Both factors can act independently to maintain a precursor in an import-competent conformation (i.e. prevent aggregation), but only MSF can act on aggregated precursors to restore solubility and import-competence [34–36]. When the MSF–preadrenodoxin complex is incubated with mitochondria, it can be co-sedimented with the mitochondria, and the interaction is mediated by the Tom70 subunit of the TOM complex [37]. Upon addition of ATP, the precursor is released and passed onto Tom20 where it is imported into the mitochondria [36,37].

Genetic screens for further factors involved in mitochondrion protein import involved have been used to search for further factors in the cytosol that might assist mitochondrial protein import. Mutants defective in Ydj1p were originally discovered in one such screen [29,38]. Other screens have identified candidate factors that have subsequently been shown to effect import indirectly, primary through effects on transcription, translation and turnover of precursor proteins.

2.6. MFT1

A LacZ fusion protein targeted to the mitochondria matrix accumulates in the inner membrane space and titrates the function of Yme1p chaperone, causing a disruption in mitochondrial function due to a decrease in the correct assembly of the electron transport chain [39,40]. Four mutants were isolated which could suppress this defect [41]. The first of these, *MFT1*, encodes a 54-kDa protein subunit of a large soluble oligomeric complex. The isolated subunit (Mft1p/Mft52p) can bind basic, amphipatic targeting sequences and was a candidate targeting sequence-binding protein [42]. However, the partners of Mft1p—Tho2p, Hpr1 and Thp2p—and the localisation of the oligomeric complex in both the cytosol and nucleus revealed the function of Mft1p to be in stable expression of the $F_1\beta$–LacZ fusion protein. The THO complex, as it has been named, is required for efficient transcription and stability of LacZ transcripts. Both the Hpr1p and Tho2p subunits of the particle have basic, amphipathic sequences that might provide for subunit–subunit interactions with Mft1p [43].

2.7. MDPs

Mod5p-I is a tRNA isopentenyltransferase distributed between the cytosol and the mitochondria, and a genetic screen was used to identify factors promoting the import of those precursor molecules in mitochondria [44]. Four such *mdp* mutants have been isolated, and each seems to indirectly influence Mod5p import: *MDP1* encodes Rsp5p, a ubiquitin-protein ligase; *MDP2* corresponds to the structural gene for actin; and *MDP3* encodes Pan1p, a protein involved in both organisation of the actin cytoskeleton

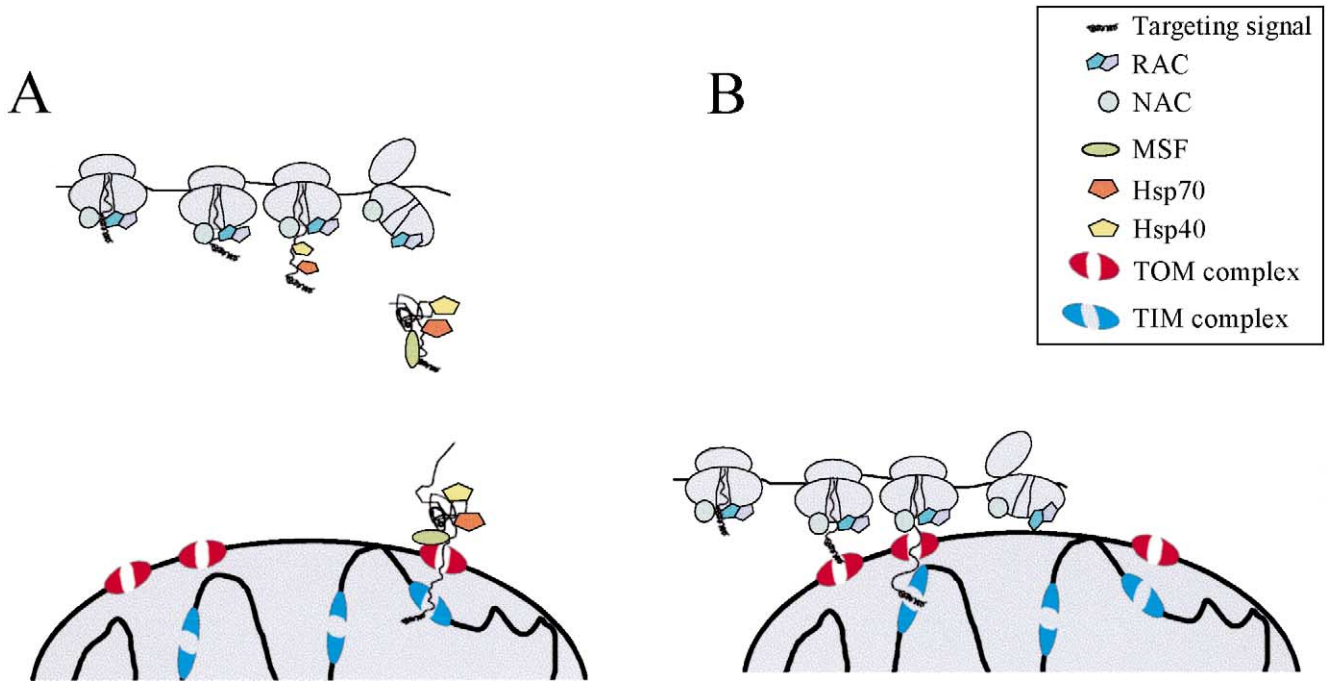

Fig. 1. Delivery of nascent polypeptides to the mitochondrial surface. Most mitochondrial proteins are synthesised on cytosolic ribosomes and large proportion of molecules will be synthesised before they encounter a mitochondria. (A) In this case, factors like Hsp70 and Hsp40 can bind to the precursor while it is still being translated to prevent aggregation, misfolding or proteolysis. Once synthesis is completed, the nascent precursor can also be bound by MSF to aid in its import. (B) If ribosome synthesising a mitochondrial precursor encounters a mitochondrion during synthesis, the precursor can import co-translational as precursor is held in an import-comptent state by ribosome-associated factors NAC and RAC.

and initiation of translation on cytoplasmic ribosomes [45,46].

2.8. What is the likely sequence of events between the initiation of protein synthesis and translocation of a precursor protein across the mitochondrial membranes?

After export into the cytoplasm mRNA becomes translationally active, and if the mRNA encodes a mitochondrial precursor protein, early passes of ribosomes would generate complete precursors that will encounter the mitochondrial surface posttranslationally (Fig. 1A). Molecular chaperones such as Hsc70 and MSF can bind the nascent precursor molecules and prevent them from folding or aggregating. There is no evidence that these precursor–chaperone complexes are directed to the mitochondrial surface, but in chance encounters with the mitochondrial surface the precursor molecule is surrendered by the chaperone to bind productively to the TOM translocase.

Given the dynamic nature of the cytoplasm including the mitochondria themselves, and at least in some cases contributions from the 3′ untranslated sequences of the mRNA, translationally active polysomes will eventually come to close proximity with mitochondria. Any molecule of precursor engaging the TOM complex on the mitochondrial surface will provide for co-translational import of all subsequent precursor molecules made from that molecule of mRNA (Fig. 1B).

Ribosome-associated factors, like NAC and RAC, might promote import by influencing the way in which the amino-terminal mitochondrial targeting sequence is presented to the TOM complex during these latter rounds of translation. We do not yet know if they do this actively or passively: by direct binding of the targeting sequence to induce an import-competent conformation, or by simply occluding a surface on the ribosome to which mitochondrial targeting sequences might otherwise interact nonproductively. Further unanswered question is whether there are any physical interactions between the ribosomes or any of the molecular chaperones with proteins on the mitochondrial surface.

Clearly, the delivery of a large select set of nascent polypeptides to the mitochondrial surface is an important problem for eukaryotic cells. The diverse set of factors on hand to negotiate this process prior to, during and after protein synthesis ensures efficient and effective protein translocation import into the mitochondria.

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