

Stress proteins and mitochondrial protein import

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Introduction

Many proteins have to cross membranes to get from their site of synthesis, usually the cytosol, to their functional destination in various intracellular compartments (the organelles) (Wickner and Lodish, 1985). Recent research is directed towards elucidating the molecular mechanisms of translocation of proteins across membranes that are naturally impermeable to macromolecules. We thereby have focused on the biogenesis of mitochondrial proteins.

Mitochondria import the vast majority of their proteins from the cytosol (Attardi and Schatz, 1988; Hartl and Neupert, 1990; Pfanner and Neupert, 1990). Precursor proteins are recognized by receptors on the mitochondrial surface and are imported at sites of close contact between outer and inner membranes. In the inner mitochondrial subcompartment, the matrix, the amino-terminal signal sequences (presequences) of the precursors are proteolytically removed by the enzyme processing peptidase.

The process of membrane translocation of mitochondrial precursor proteins is not really understood. We know that various energy sources, ATP in the cytosol and matrix and the electrical membrane potential across the inner membrane, are required. Moreover, precursor proteins are unfolded prior to translocation and are refolded in the matrix. Several stress proteins (heat shock proteins, hsp) seem to play critical roles with regard to translocation and modifying the conformation of precursor proteins.

Translocation competence of mitochondrial precursor proteins

The uptake of precursor proteins by mitochondria occurs predominantly in a posttranslational man-

ner, i.e., fully synthesized precursor proteins are transported into the organelle. This implies that the precursor proteins in the cytosol have to adopt conformations which are compatible with the subsequent unfolding of the polypeptide chain, an essential prerequisite for membrane translocation. In particular, aggregation or misfolding of precursor proteins must be prevented.

Upon the discovery that cytosolic heat shock proteins of 70 kd (ct-hsp70s) are involved in protein import (Deshaies et al., 1988; Murakami et al., 1988; Randall and Shore, 1989), it was generally assumed that ct-hsp70s participate in maintaining a translocation-competent conformation of precursor proteins. This is based on the observations that hsp70s seem to interact with (partially) unfolded proteins, thereby stabilizing a loosely folded conformation and preventing misfolding or aggregation of proteins (Pelham, 1988; Rothman, 1989). The release of proteins from hsp70s requires hydrolysis of ATP. Indeed protein import into mitochondria was found to require ATP in the cytosol and the ATP-dependence could be correlated with the acquisition of transport-competence of the precursor proteins (Pfanner et al., 1987, 1988; Verner and Schatz, 1987; Chen and Douglas, 1988).

It has to be emphasized that cytosolic hsp70s and ATP are probably not directly involved in the unfolding of precursor proteins. We found that some precursor proteins with tightly, yet correctly folded domains did not require the addition of ATP for unfolding and membrane translocation, in contrast to the numerous precursor proteins with an intermediate degree of folded structure (Pfanner et al., 1990). As correctly folded domains obviously have a very low tendency for misfolding or aggregation, they may not depend on the protective effect of hsp70s. In molecular terms, tightly folded domains probably do not expose sequences that would bind to hsp70s (Rothman,

1989), and thus there is no need for ATP to release hsp70s. It is concluded that ct-hsp70s stabilize the conformation of precursor proteins that would otherwise form improper interactions or structures in the cytosol. The actual unfolding of precursors may be performed by the membrane-bound import apparatus of mitochondria.

Mitochondrial hsp70 and protein transport through contact sites

Contact sites between outer and inner membranes are the main entry gate of mitochondria. This was demonstrated by the reversible arrest of precursor proteins in contact sites in a two-membrane spanning manner (Schleyer and Neupert, 1985; Schwaiger et al., 1987). A recent study confirmed the implication that a polypeptide chain spanning two membranes is largely unfolded. Hybrid proteins with a tightly folded carboxyl-terminal domain and an amino-terminal portion of variable length were accumulated in mitochondrial contact sites such that the folded domain remained on the cytosolic side of the membranes. We thereby found that about 50 amino acid residues were sufficient to span both mitochondrial membranes (Rassow et al., 1990). Considering the distance across two protein-rich membranes, this result suggests that the polypeptide chain has to be rather extended.

Where does the energy for an extensive unfolding of the polypeptide chain come from? The electrical potential across the inner membrane is required for the initial transfer of an amino-terminal portion of the precursor protein into the matrix. The completion of transport (including unfolding) of the major part of the precursor protein, however, can take place in the absence of the membrane potential (Schleyer and Neupert, 1985; Rassow et al., 1989). As discussed above, cytosolic ATP can also be excluded as the possible energy source. The precursor protein in transit through contact sites seems to interact with the mitochondrial hsp70 (mt-hsp70, also termed Ssc1p) that is located in the matrix (Kang et al., 1990). In a yeast mutant that was defective in mt-hsp70 in a temperature-sensitive manner, precursor proteins could be inserted into contact sites such that the presequence reached the matrix space. However, the completion of transport, in-

cluding the unfolding of the polypeptide chain on the cytosolic side, was impaired at the non-permissive temperature, suggesting that functional mt-hsp70 was necessary for unfolding and membrane translocation of the precursor protein. Indeed, the artificial unfolding of a precursor protein by a pretreatment with 7 M urea allowed a partial circumvention of the transport defect in this mutant mitochondria (Kang et al., 1990). We propose a hypothesis in that one or more mt-hsp70s bind to precursor proteins emerging on the matrix side of the inner membrane and "pull" the precursor proteins towards the inside of mitochondria, thereby facilitating the unfolding reaction on the cytosolic side. The free energy of binding of a precursor protein to mt-hsp70 may thus provide the driving force for unfolding and vectorial translocation of the polypeptide. ATP would then be indirectly required to allow release of the imported precursor protein from mt-hsp70, setting the heat shock protein free for new rounds of transport.

Refolding of imported proteins

Imported proteins do not refold spontaneously. Mt-hsp70 probably keeps them in an unfolded conformation (Kang et al., 1990) and eventually transfers them to a heat shock protein of 60 kd in the matrix (hsp60). Hsp60 seems to act as "foldase", catalyzing the folding of polypeptides in an ATP-dependent process (Ostermann et al., 1989). At low levels of ATP, precursor proteins can be accumulated on the surface of hsp60 in an unfolded conformation. Addition of ATP promotes the folding of the polypeptide chain in association with hsp60. It is not known whether further components in the matrix participate in this folding reaction. The release of the proteins from hsp60 requires additional protein factors and ATP (Ostermann et al., 1989). This function of hsp60 in folding imported proteins is an essential prerequisite for the assembly of proteins into multi-subunit complexes as was found with a yeast mutant that was defective in hsp60 in a temperature-sensitive manner (Cheng et al., 1989).

Conclusions and perspectives

Stress proteins, cytosolic and mitochondrial

hsp70s and mitochondrial hsp60, are important components in the complex machinery that imports nuclear-encoded precursor proteins into mitochondria. Hsp70s and hsp60 are involved in the various processes that involve modifying or stabilizing the conformation of precursor proteins. The identification and characterization of these stress proteins should now allow a molecular analysis of the interaction with precursor proteins. Since the stress proteins of the hsp70- and hsp60-families are present in various intracellular compartments, they most likely play critical roles in practically all processes that involve unfolding, translocation, folding or assembly of newly synthesized proteins (for overview, see Ellis and Hemmingsen, 1989; Rothman, 1989). Investigations on the role of stress proteins in mitochondrial protein import should thus reveal mechanisms that are most likely of relevance for the biogenesis of proteins and cell organelles in general.

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