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The energetics of protein import into mitochondria

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Mitochondrial DNA encodes very few proteins; most mitochondrial proteins are therefore synthesized by the nucleo-cytoplasmic system and imported into the mitochondria [1]. During the past decade, many of the signals that target proteins to different intramitochondrial locations have been characterized [2–5]. More recently, there has also been considerable progress in identifying components of the import machinery [2,3]. These studies have shown that protein import into mitochondria is a multistep process that requires components in the cytosol, on the mitochondrial surface and within the mitochondria.

Protein import into mitochondria also requires energy [5,6]. Proteins targeted to the matrix follow a common pathway that requires an electric potential across the inner membrane, as well as ATP both in the cytosol and in the matrix. Proteins targeted to the outer membrane, the intermembrane space, or the inner membrane are imported by a variety of pathways that differ in their energy requirements. These distinct energy requirements have been used to characterize the various sorting pathways, and to identify components of the import machinery.

The 'matrix pathway' is the best-characterized import route. A matrix-targeted protein is usually synthesized as a precursor with a transient amino-terminal matrix-targeting sequence. This precursor binds to one of several antifolding proteins in the cytosol which include members of the 70 kDa class of chaperone proteins. These proteins prevent tight folding of the precursor. Release of the precursor from the chaperone proteins probably requires hydrolysis of ATP. The released precursor is recognized by receptors on the mitochondrial surface; it then passes through the import channel in the outer membrane and inserts its matrix-targeting signal across the inner membrane. This insertion requires an electric potential across the inner membrane, as well as a proteinaceous import machinery in that membrane. On the matrix side of the inner membrane, the precursor's amino-terminal region binds to a mitochondrial 70 kDa chaperone protein (termed mhsp70), and the entire precursor is then pulled into the matrix. Transport across the inner membrane requires ATP in the matrix, and may involve successive cycles of binding and release of the precursor by mhsp70. The imported precursor is released from mhsp70 as an incompletely folded polypeptide chain [7]; folding is usually completed by reversible interaction with hsp60, the mitochondrial homolog of bacterial groEL [8]. This reversible interaction requires hydrolysis of ATP by hsp60.

Import into the matrix thus involves at least three ATP-requiring steps, each of which is mediated by a chaperone protein (Fig. 1).

The ATP requirement outside the mitochondria can be bypassed by denaturing the precursor with urea before presenting it to mitochondria (Wachter, C. and Glick, B. unpublished data; see also Ref. 9). A cotranslational import mode would probably also not require extramitochondrial ATP, except for protein synthesis itself. In addition, import of some matrix-targeted precursors is inherently independent of extramitochondrial ATP [10,11]. These precursors can apparently maintain an import-competent conformation without the aid of antifolding proteins, or can be unfolded by acidic phospholipids on the mitochondrial surface in an ATP-independent reaction [12].

When a matrix-targeted precursor is imported into mitochondria that maintain a potential across the inner membrane, but that have been depleted of matrix ATP, the precursor is transported across the outer membrane, and its amino-terminal portion inserts into the inner membrane Thus, the precursor becomes stuck between the two membranes [13,14]. This 'ATP-depletion intermediate' can then be chased into the matrix if the ATP level in the matrix is restored.

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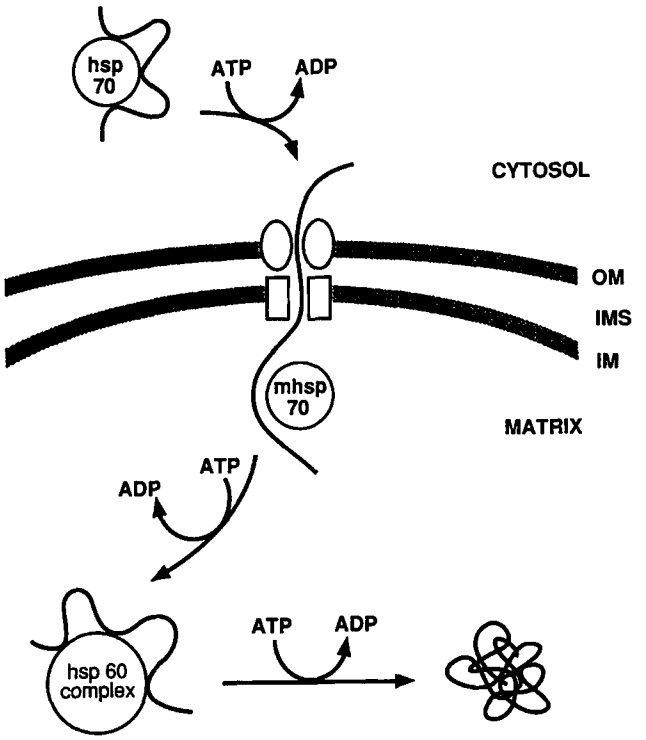

Fig. 1. The three ATP-dependent steps of protein import into the mitochondrial matrix. Wavy line, matrix-targeted precursor protein; hsp70 and mhsp70, cytosplasmic and mitochondrial 70 kDa heat-shock proteins; OM, outer membrane; IMS, intermembrane space; IM, inner membrane; hsp60 complex, mitochondrial homolog of *E. coli* groEL; ellipses and rectangles, subunits of the translocation systems in the outer and inner membrane.

Insertion of proteins into the outer membrane requires extramitochondrial ATP and (presumably) cytosolic antifolding proteins, but bypasses the requirements for intramitochondrial ATP, a membrane potential, and intramitochondrial components of the import machinery [5].

Protein targeting to the intermembrane space occurs by several quite different routes. One of the simplest is that followed by cytochrome *c*. This protein is initially made as the heme-free apoprotein which appears to insert directly into the lipid bilayer of the outer membrane. The protein is then pulled across the outer membrane by the enzyme-catalyzed addition of heme in the intermembrane space. This import route seems to be independent of ATP or a membrane potential [2]. Several other intermembrane space proteins are first transported across the outer membrane, and then become anchored to the outer face of the inner membrane by their transient presequences. After removal of the presequence, these proteins may remain bound to the outer face of the inner membrane, or they may be released into the intermembrane space. Such a 'stop-transfer' pathway has been demonstrated for the yeast proteins cytochrome c_1 and cytochrome b_2 [15]. These proteins are synthesized as precursors whose transient presequences contain a bipartite targeting signal: the amino-terminal part of the presequence resembles a matrix-targeting signal, and the carboxy-terminal part contains a sorting signal that prevents transport of the mature domain across the inner membrane [4,5]. Import of cytochrome c_1 to the intermembrane space requires extramitochondrial ATP and a potential across the inner membrane, but not ATP in the matrix (Wachter, C. and Glick, B. unpublished data).

Import of proteins into the inner membrane may occur by a 'direct' route, or by a 'detour' route. In the direct route, the protein is transported across the outer membrane and inserted directly into the inner membrane. This route, which is exemplified by the ADP/ATP translocator, requires extramitochondrial ATP and a potential across the inner membrane, but not ATP in the matrix (Ref. 16; Wachter, C. and Glick, B., unpublished data). In the detour route, the protein is first completely transported into the matrix, and then assembles with its partner subunits into the inner membrane from the matrix side. This route requires ATP both outside and inside the mitochondria, and also a potential across the inner membrane; it is exemplified by subunit IV of yeast cytochrome oxidase (Ref. 17; Wachter, C. and Glick, B., unpublished data).

Current information about the mitochondrial protein sorting pathways thus agrees well with the known energy requirements of import into the different mitochondrial compartments. This information should be useful for identifying novel components of the import machinery. For example, we have recently crosslinked the 'ATP-depletion' intermediate mentioned above to specific proteins of the inner membrane (Scherer, P. and Manning-Krieg, U., unpublished data).

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