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# Protein Synthesis and Targeting in Yeast

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# Intra-mitochondrial sorting of precursor proteins

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## Introduction

With the exception of a limited amount of protein synthesis in mitochondria and chloroplasts, the majority of eukaryotic protein synthesis occurs in the cytoplasm. Consequently those proteins destined for the various cellular subcompartments other than the cytosol must be correctly targeted to their functional location. Many precursor proteins are thus translocated into or across biological membranes in order to reach their functional destination. This includes protein translocation into the endoplasmic reticulum, mitochondria, chloroplasts and peroxisomes.

The vast majority of mitochondrial proteins are encoded for by the cell nucleus and are synthesized in the cell cytosol as precursor proteins. These precursors are imported into mitochondria in a post-translational manner along a number of distinct, but sometimes overlapping pathways. Mitochondria are divided into four subcompartments outer membrane (OM), intermembrane space (IMS), inner membrane (IM) and matrix (M), each equipped with a specific set of proteins. Hence precursor proteins must contain the necessary information to target them not only to mitochondria but also for their correct submitochondrial sorting. Our knowledge of mitochondrial protein import has increased over the past years due to a number of detailed *in vitro* studies using mainly *Saccharomyces cerevisiae* and *Neurospora crassa* as model systems (for reviews see Hartl & Neupert, 1990; Baker & Schatz, 1991).

In most organisms, lack of mitochondrial function is lethal to the cell. *S. cerevisiae,* however, can survive with defective respiratory chain and oxidative phosphorylation because it can use fermentable carbon sources for energy production. This ability to survive with defective mitochondria has rendered *S. cerevisiae* an excellent organism for the study of mitochondrial biogenesis. Furthermore, the genetic manipulation possibilities are greater in yeast than in other organisms such as *N. crassa*, and this has resulted in the identification of a number of mutants which have proved to be powerful in the analysis of mitochondrial protein import (for reviews see Grivell, 1989, Tzagoloff & Dieckmann, 1990, Bolotin-Fukuhara & Grivell, 1992).

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## The mitochondrial import machinery

Mitochondrial precursor proteins contain targeting sequences which in the majority of cases are positively-charged, amino-terminal cleavable presequences. Not all precursors contain such presequences however, this includes all of the outer membrane proteins and some of those of the IMS. The first stage of mitochondrial protein import involves the interaction of precursors with surface receptor proteins on the outer surface of mitochondria. Recent studies using *N. crassa* have lead to the identification of the first mitochondrial import receptors, namely, MOM19 and MOM72 (Mitochondrial Outer Membrane proteins of 19kD and 72kD molecular weights, respectively (Söllner et al., 1989, 1990). MOM19 appears to act as the receptor for the majority of precursors tested to date, whilst MOM72 seems to be primarily involved in the binding of the inner membrane protein the ADP/ATP translocator. The yeast homolog of MOM72, termed MAS70, has also been identified and appears to have a broader specificity (Hines et al., 1990; Steger et al., 1990). Coimmuneprecipitation studies have indicated that MOM19 and MOM72 are present in a multi-subunit complex that catalyses both the recognition and the insertion of precursors into the outer membrane (Söllner et al., 1992). This outer membrane complex known as GIP, the General Insertion Pore (Pfaller et al., 1988). One component of this GIP complex, MOM38 is believed to be part of the OM translocation site (Kiebler et al., 1990). ISP42, the yeast equivalent of MOM38 has also been identified and has been shown to be essential for growth (Baker et al., 1990). MOM7, MOM8 and MOM30 are other components of the *N. crassa* GIP complex which have been identified, however, their functions to date are unknown.

Translocation of proteins across the inner membrane occurs at areas where the inner membrane is adjacent to the outer membrane. It has been estimated that *in vivo* most of the outer membrane is in close proximity to the inner membrane (Pfanner et al., 1992). Translocation across the inner membrane requires the presence of a membrane potential. Although the insertion of precursors into the outer membrane does not require a membrane potential per se, many proteins destined to cross the inner membrane cannot stably insert into the outer membrane in the absence of $\Delta\Psi$.

Passage of proteins across the inner membrane is also thought to occur through a proteinaceous pore, the components of which are not as yet identified. In addition to a $\Delta\Psi$, translocation through the inner membrane requires the cooperation of Hsp70 in the mitochondrial matrix (see below). The matrix-targeting presequences are proteolytically cleaved off, a reaction catalyzed by the matrix localized processing peptidase (MPP) in association with the processing enhancing protein (PEP) processing peptidase in the matrix. Folding of proteins in the matrix is mediated by Hsp60 in an ATP-dependent manner (see below).

*Role of mitochondrial Hsp70 in protein translocation and folding*

Translocation of proteins across the outer and inner membrane requires that precursor proteins have a loosely folded conformation. Cytosolic Hsp70 proteins have been implicated in playing a role in the maintenance of precursor in an unfolded conformation competent for translocation. As the precursor on transit through the inner membrane becomes exposed to the matrix, it forms an association with the mitochondrial Hsp70 (mt-Hsp70), also termed Ssc1p and is encoded for by the SSC1 gene. Interaction with mt-Hsp70 apparently is directly required for complete translocation into the matrix. In a temperature-sensitive yeast mutant affecting the gene coding for Ssc1p, the transfer of precursor proteins into mitochondria was shown to be defective (Kang et al., 1990). Precursor polypeptides were arrested during translocation spanning outer and inner membranes at contact sites. If however, the precursor proteins were artifically denatured with urea beforehand, import into mitochondria appreared to be mt-hsp70 independent. However, the precursor proteins imported under these conditions remained in a highly protease-sensitive, incompletely folded conformation. Therefore, it was concluded that the mitochondrial Hsp70 apparently has a dual role in translocation and folding of imported protein. Folding requires the transfer of the newly translocated polypeptides from mt-Hsp70 to Hsp60 (see below), a step that is blocked in the *ssc1* mutant. The energy resulting from binding the extended amino-terminus of the precursor protein to mt-Hsp70 is thought to be utilized to successively unfold parts of the precursor still outside the mitochondria. Release of mt-Hsp70 requires ATP hydrolysis in the matrix. Multiple molecules of mt-Hsp70 could bind to the traversing chain, thereby assisting it through the membrane.

*Role of Hsp60 in the folding and assembly of proteins imported into the matrix*

Evidence for the function of Hsp60 in protein assembly came from analysis of the temperature sensitive lethal yeast *mif4*, which is defective in the MIF4 gene coding for Hsp60. Proteins such as the ß subunit of the $F_1$-ATPase or the trimeric enzyme ornithine transcarbamylase are imported normally by the mutant mitochondria and are proteolytically processed, but cannot assemble into oligomeric complexes (Cheng et al., 1989). The unassembled subunits have a tendency to aggregate in the matrix compartment. The Hsp60 14-mer was also demonstrated to be required for not only its own assembly but also for the assembly of certain proteins which are targeted to the IMS, such as cytochrome $b_2$, which are imported via the matrix. Thus it is suggested Hsp60 plays a general role in protein assembly processes *in vivo*. A biochemical analysis of the interaction between imported proteins and mitochondrial Hsp60 revealed that the basic role of this chaperonin probably lies in mediating the folding of monomeric polypeptide chains. Using protein import into mitochondria as an experimental system to study protein folding, it could be demonstrated that the soluble, monomeric protein dihydrofolate reductase (DHFR) does not fold spontaneously when

imported into mitochondria. Folding was demonstrated to occur in an ATP-dependent reaction mediated hy Hsp60 (Ostermann et al., 1989).

## Sorting of proteins to the mitochondrial IMS

Precursor proteins targeted to the mitochondrial matrix are imported along the general pathway described above. Other precursors destined for the other mitochondrial subcompartments initiate import along this general pathway, however, become deviated off at different points, depending on their final destination. For example porin, an outer membrane protein like the matrix-targeted proteins, uses the MOM19 receptor for initial mitochondrial recognition and binding and becomes inserted into the outer membrane in a GIP-dependent manner. Porin then becomes directly assembled into its characteristic trimeric structure in the outer membrane. Hence import and assembly of outer membrane proteins does not require the action the downstream components of the import machinery in the inner membrane and matrix.

The import of precursor proteins into the IMS represents the most complex situation for submitochondrial sorting. At least three distinct mechanisms of sorting to the IMS are known. The simplest pathway is taken by cytochrome *c*, where the precursor is imported directly across the outer membrane by a mechanism which is unique to this precursor (Stuart & Neupert, 1990). A second mechanism which has recently been shown to exist is the pathway taken by cytochrome *c* heme lyase (CCHL), which like porin uses the receptor/GIP complex (Lill et al., 1992). The third and probably most complex sorting pathway involves a multistep import pathway to the IMS (Hartl & Neupert, 1990).

*Import of cytochrome c*

The cytochrome *c* pathway differs markedly from the general route taken by the majority of other imported proteins, as it is characterized by the lack of involvement of surface receptors, GIP and energy requirements such as ATP hydrolysis and a membrane potential. Cytochrome *c* is synthesized as a precursor protein termed apocytochrome *c* and does not contain a cleavable targeting presequence (Stuart at al., 1987). No protease-sensitive components exist on the mitochondrial surface to mediate the binding of apocytochrome *c*. Instead, it is believed that due to the ability of apocytochrome *c* to spontaneously insert into lipid bilayers, it can become partially inserted into the outer membrane without the requirement of receptors etc. Upon insertion, apocytochrome *c* is recognized and binds in a complex with CCHL (Nicholson et al., 1988). Hence it is believed that CCHL acts as a specific binding site *in lieu* of a surface receptor. CCHL is the enzyme responsible for catalyzing the covalent attachment of heme to apocytochrome *c* and displays a requirement for NADH and flavins. Translocation across the outer membrane is believed to be driven by the refolding of the cytochrome *c* polypeptide as a

result of the covalent heme addition, in a manner which does not require ATP hydrolysis (Stuart et al., 1990).

*Import of cytochrome c heme lyase*

Cytochrome *c* heme lyase is peripherally associated with the inner membrane, exposed to the IMS. Like apocytochrome *c* it is synthesized as a precursor lacking an amino-terminal cleavable presequence and consequently does not undergo cleavage upon import into mitochondria. Preliminary results suggest that an internal signal present in the central region of CCHL serves to target the precursor of CCHL to mitochondria (G. Kispal, B. Segui-Real, R. Lill & W. Neupert, in preparation). CCHL however, requires the function of the receptor/GIP complex for insertion into the outer membrane. Like the majority of precursors, CCHL appears to use the MOM19 as a receptor. In contrast to proteins targeted to the matrix, CCHL does not require the presence of an electrochemical membrane potential nor ATP hydrolysis to drive its import to the IMS. CCHL is imported directly across the outer membrane and does not traverse the inner membrane during its import (Lill et al., 1992). As CCHL does not appear to use the energy sources used by other precursor for import the question thus arises as to energetically drives the import events. One possible explanation is that the translocation of CCHL across the outer membrane is powered by the folding of the polypeptide chain upon emergence in the IMS.

*Sorting of other proteins to the IMS*

The third and probably most complex sorting pathway to the IMS exists for proteins such as cytochrome $b_2$, cytochrome $c_1$ and the Rieske Fe-S protein of the cytochrome $bc_1$ complex. Two models of the sorting events have been proposed, namely the "conservative sorting" mechanism and the "stop transfer" mechanism. The "conservative sorting mechanism" takes into account the evolutionary origin of the mitochondrion (Hartl et al., 1986, 1987; Hartl and Neupert, 1990). As is generally accepted, mitochondria have evolved from prokaryotic ancestors that were introduced into an ancestral eukaryotic host cell by an endosymbiotic event. The conservative sorting hypothesis proposes that the prokaryotic principles of membrane assembly and transport have been maintained during the evolution of the mitochondria. For example, *Rhodobacter* are probably close relatives of the endosymbiotic ancestor. Analysis of the synthesis and assembly pathway of the bacterial cytochrome $c_1$ reveals that this precursor is synthesized with a typical bacterial cleavable presequence and undergoes export from the cytoplasm across the plasma membrane to the periplasmic space. The conservative sorting hypothesis proposes that this export pathway has been conserved in mitochondria. Thus it is proposed that mitochondrial IMS proteins which follow this sorting pathway are initially targeted into the mitochondrial matrix and are subsequently exported into or across the inner

membrane. Cytochromes $b_2$ and $c_1$ are initially synthesized as precursors with a bipartite amino-terminal targeting sequence which becomes processed in two steps upon import. The first domain is a positively-charged and serves as a "matrix targeting" domain and is cleaved by the matrix-localized processing peptidase, to generate an intermediate-size species. We believe that the second domain of the presequence contains the necessary information to direct the export of the protein from the matrix to the intermembrane space. This second domain is very similar to the leader or signal sequences of bacterial and eukaryotic secretory proteins, namely it contains several positively-charged residues at the amino-terminus which are followed by a hydrophobic segment of approximately 20 residues. These positive amino acids are of particular importance, as deletion of one or more of them has recently been shown to prevent the export of cytochrome $b_2$ from the matrix to the intermembrane space (E. Schwarz, T. Seytter & W. Neupert, in preparation).

Thus it is suggested that proteins such as cytochrome $b_2$, cytochrome $c_1$ and the Rieske Fe-S protein reach the intermembrane space by export from the matrix, which resembles the pathway of protein secretion into the bacterial periplasm. Under certain import conditions where export is retarded, however, such as low temperature, these proteins can be accumulated in the matrix as intermediate-size species (Hartl et al., 1987; Stuart et al., 1990). Recent evidence suggest that the export of these proteins from the matrix can occur in concert with completion of the import step (Koll et al., 1992). Using specially designed cytochrome $b_2$-DHFR fusion proteins, it could be shown that the sites of the protein export machinery can be in very close proximity to the sites of mitochondrial protein import, namely the translocation contact sites. This was demonstrated by the accumulation of Hsp60-bound intermediates of the cytochrome $b_2$-DHFR fusion proteins which were spanning three membranes, namely the outer and inner membranes at contact sites (the site of import) and again the inner membrane at the site of export. Using different protein fusion constructs, where the length of the cytochrome $b_2$ part was varied, it could be calculated that the minimal distance between the sites of import and those of export maybe as little as 30nm.

A second model for the mechanism of sorting of these proteins to the IMS, termed the"stop-transfer hypothesis" has been proposed by Schatz and co-workers (van Loon & Schatz, 1987). According to the "stop transfer" hypothesis, the second domain of the presequence serves to arrest the protein in the inner membrane upon import of the precursor into mitochondria. Following cleavage of the matrix targeting presequence, the intermediate species would be sorted to the IMS by a lateral movement in the inner membrane. Hence unlike the conservative sorting hypothesis, the stop-transfer hypothesis would predict that whilst on transit to the IMS, the mature part of the protein would never become exposed to the mitochondrial matrix. However, both sorting models would predict that the intermediate-size species would finally accumulate in the intermembrane space, membrane bound, prior to its final processing event to generate the mature-size species. Evidence in favour of the "stop

transfer" model was recently published by Glick et al., where they failed to observe both intermediates accumulated in the matrix and a dependence of matrix ATP for correct sorting to the IMS (Glick et al., 1992). As explained above, however, if initiation of export of precursors can occur prior to completion of import, intermediates in the matrix would not be observed. Futhermore, only under certain experimental conditions where the kinetics of import are greater than those of export, (i.e. low temperature, or import of urea denatured precursors) can one accumulate such sorting intermediates in the matrix. As these are kinetic intermediates, they are labile and easily chased to their final location, exposed to the IMS whilst associated with the inner membrane (via the remaining uncleaved part of the presequence in the case of cytochrome $b_2$), a location which is also predicted for the intermediate-size species along the stop-transfer sorting pathway.

ATP hydrolysis in the matrix is required for the function of Hsp70 and Hsp60. In addition Glick et al., presented evidence which suggested that a fusion protein p$b_2$(167)-DHFR, consisting of 167 amino acids of cytochrome $b_2$ fused to mouse dihydrofolate reductase (DHFR), did not appear to require matrix ATP hydrolysis, and hence concluded that it was not being sorted via the matrix. This finding is in contrast to previously published results where authentic cytochrome $b_2$, under similar experimental conditions was shown to require matrix ATP, (Hwang & Schatz, 1989). The discrepancy here suggests that the apparent matrix ATP requirement reflects the IMS-targeted precursor protein being studied rather than the sorting pathway being taken. This strongly argues that the lack of an observable matrix ATP requirement cannot be used as an criterion in favour or against one sorting pathway or the other. Furthermore, following a study of the matrix ATP requirements for the IMS sorting of a series of cytochrome $b_2$-DHFR of increasing length, it would appear that longer precursor proteins display a greater requirement for matrix ATP than the smaller ones. This difference is most likely related to the level of involvement of Hsp70 that is required for the matrix import step. This seems to be reduced for the smaller precursors, as it appears that the export process, which can occur in concert with import, is sufficient to power the completion of import (A. Gruhler, W. Voos, unpublished results).

Evidence that Hsp60 could be involved in protein sorting to the IMS came from analyzing the Hsp60-deficient yeast mutant *mif4*. The mutant accumulated incompletely processed forms of cytochrome $b_2$ and of the Rieske Fe/S protein, which is believed to follow a similar route to the IMS. Recent evidence suggested a role for Hsp60 in maintaining precursor proteins imported into the matrix in an open conformation competent for export across the inner membrane (Koll et al., 1992). In contrast, Glick et al., fail to observe a requirement for Hsp60 for the sorting of cytochrome $b_2$ to the IMS. The cause for the discrepancy is, however, unclear and further experiments are clearly needed to resolve this situation. One possibiltiy is that maybe the requirement of Hsp60 is not absolute and depending on the import conditions used its function can be circumvented. One could imagine this to be the case under conditions

where export is initiated prior to the completion of the import event, especially in the case of smaller precursor proteins.

Following export across the inner membrane, the proteins undergo a second proteolytic cleavage to generate the mature-size species and the proteins then assemble into their functional oligomeric complexes. An intermembrane space protease IMP1, which is involved with the cleavage of the second domain (the "sorting sequence") of the presequences of some of the IMS proteins has been identified and cloned. IMP1 is a membrane bound enzyme which is thought to be part of a multi-subunit complex and it was shown to be structurally related to the bacterial leader peptidase (Wolfe et al., 1983; Schneider et al., 1991; Behrens et al., 1991). The similarity of IMP1 to the bacterial peptidase is further support for the conservative sorting hypothesis which emphasizes the resemblance between the export of proteins from the matrix to the main pathway of protein secretion into the bacterial periplasm.

Initially the conservative sorting hypothesis was described for a subset of IMS proteins, but now following a recent study it has been extended to include a subset of inner membrane proteins. Proteins of the inner membrane which are synthesized as precursor proteins with cleavable amino-terminal extensions, such as the subunit 9 of the Fo-ATPase, have been shown to be initially imported into the mitochondrial matrix and following an interaction with Hsp60 become retranslocated into the inner membrane (Mahlke et al., 1990).

## Summary and future perspectives

The mitochondrial matrix may be envisaged as a dynamic milieu, as it serves as a temporary depot for many proteins that are on transit to the various submitochondrial locations. In addition to those proteins with their functional location in the matrix, some nuclear-encoded proteins destined for the inner membrane and intermembrane space, are initially imported into the matrix prior to subsequent sorting. Furthermore, proteins encoded by the mitochondrial genome are also synthesized in the matrix and from there are sorted to the IM and IMS. The mitochondrial matrix, hence, is the scene for many events that have important consequences for the correct sorting and assembly of precursor proteins encoded by both genomes.

A consequence of the "conservative sorting" pathway of proteins to the IMS, is that the inner mitochondrial membrane must contain a separate translocation machinery for the export of proteins into and across the inner membrane. An open question of course is whether the mitochondrially-encoded precursor proteins use the same export machinery as those nuclear proteins which undergo export from the matrix. The identification and characterisation of such a translocation machinery is thus of utmost importance and will the main focus of our attention in the next few years.

## References


Baker, K. & Schatz, G, (1991). *Nature*, **349**, 205-208.

Baker, K.P., Schaniel, A., Vestweber, D. & Schatz, G. (1990). *Nature*, **348**, 605-609.

Bolotin-Fukahara, M. & Grivell, L.A. (1992). *Antonie van Leewenhoek*, **62**, 131-153.

Cheng, M.Y., Hartl., F.-U., Martin, J., Pollock, R.A., Kalousek, F., Neupert, W., Hallberg, E.M., Hallberg, R.L. & Horwich, A.L. (1989). *Nature*, **337**, 620-625.

Glick, B., Brandt, A., Cunningham, K., Müller, S., Hallberg, R. L. & Schatz, G. (1992). *Cell*, **69**, 809-822.

Grivell, L.A. (1989). *Eur. J. Biochem.*, **182**, 477-493.

Hartl., F.-U. & Neupert, W. (1990). *Science*, **247**, 930-938.

Hwang, S.T. & Schatz, G. (1989). *Proc. Natl. Acad. Sci.*, **86**, 8432-8436.

Hines, V., Brandt, A., Griffiths, G., Horstmann, H., Brütsch, H. & Schatz, G. (1990). *EMBO J.*, **9**, 3191-3200.

Kang, P.J., Ostermann, J., Shilling, J., Neupert, W., Craig, E. A. & Pfanner, N. (1990). *Nature*, **348**, 137-142.

Kiebler, M., Pfaller, R., Söllner, T., Griffiths, G., Horstmann, H., Pfanner, N., & Neupert, W. (1990). *Nature*, **348**, 610-617.

Lill, R., Stuart, R.A., Drygas, M., Nargang, F.E. & Neupert, W. (1992). *EMBO J.*, **11**, 449-456.

Lill, R., Hergersberg, C., Schneider, H., Söllner, T., Stuart, R.A., & Neupert, W. (1992). in "Membrane Biogensis and Protein Targeting", eds., W. Neupert, & R. Lill, Elsevier, Amsterdam.

Mahlke, K., Pfanner,N., Martin, J., Horwich, A., Hartl, F.-U. & Neupert, W. (1990). *Eur. J. Biochem.*, **192**, 551-555.

Nicholson, D.W., Hergersberg, C. & Neupert, W. (1988). *J. Biol. Chem.*, **263**, 19034-19042.

Ostermann, J., Horwich, A.L., Neupert, W. & Hartl., F.-U. (1989). *Nature*, **341**, 125-130.

Pfaller, R., Steger, H., Rassow, J., Pfanner, N. & Neupert, W. (1988). *J. Cell. Biol.*, **107**, 2483-2490.

Pfanner, N., Rassow, J., van der Klei, I., & Neupert, W., (1992). *Cell*, **68**, 999-1002.

Söllner, T., Griffiths, G., Pfaller, R., Pfanner, N. & Neupert, W. (1989). *Cell*, **59**, 1061-1070.

Söllner, T., Pfaller, R., Griffiths, G., Pfanner, N. & Neupert, W. (1990). *Cell*, **62**, 107-115.

Söllner, T., Rassow, J., Wiedmann, M., Schlossmann, J., Keil, P., Neupert, W. & Pfanner, N. (1992). *Nature*, **355**,

Steger, H., Söllner, T., Kiebler, M., Dietmeier, K.A., Pfaller, R., Trülzsch, K.S., Tropschug, M., Neupert, W. & Pfanner, N. (1990). *J. Cell. Biol.*, **111**, 2353-2364.

Stuart, R.A. & Neupert, W. (1990). *Biochimie*, **72**, 115-121.

Stuart, R.A., Neupert, W. & Tropschug, M. (1987). *EMBO J.*, **6**, 2131-2137.

Stuart, R.A., Nicholson D.W. & Neupert, W. (1989). *Cell*, **60**, 31-43.

Stuart, R.A., Nicholson D.W., Wienheus, U. & Neupert, W. (1990). *J. Biol. Chem.*, **265**, 20210-20219.

Tzagoloff, A. & Dieckmann, C.L. (1990). *Microbiol. Rev.* **54**, 211-225.

van Loon, A.P.G.M. & Schatz, G. (1987). *EMBO J.*, **6**, 2441-2448.