

Review Article

An Insight into Import and Sorting of Chloroplast and Mitochondrial Proteins

M.S. Nimmy^{1*}, Vinod Kumar² and Arpita Sharma³

¹National Institute on Plant Biotechnology, New Delhi, India

²Bihar Agricultural University, Sabour, India

³Department of Botany, Maa Bharti P. G. College, University of Kota, Rajasthan, India

*Corresponding author

ABSTRACT

Chloroplasts are the double membraned light harvesting organelles present in majority of photosynthetic land plants descended from a bacterial ancestor. Most of the proteins that function inside this organelle are synthesized in the cytosol and then imported through the membrane boundaries post-translationally with the aid of transport machineries that are present in the organelle's outer and inner envelope membranes which termed TOC and TIC, respectively. The other double walled cell organelle mitochondria also harbour similar protein translocation machineries on its membranes. Most of the mitochondrial proteins are encoded by the nuclear genome synthesized in the cytosol and then imported into the organelle by a multistep process coordinated by specialized translocator proteins present on both the outer and inner membranes of it. In this review, we highlight the importance of understanding this complex process protein import into both chloroplast and mitochondria with the help of translocator proteins and how this process is regulated.

Keywords

Chloroplast,
Mitochondria,
Protein transport

Introduction

Mitochondria and chloroplasts are unique cellular organelles having double membraneous structure and semiautonomous in nature. Chloroplasts are photosynthetic organelles that are hypothesized as evolved as result of an endosymbiotic event which occurred ~1.5 billion years ago. Even though they possess their own genetic material most of the proteins required by these organelles are encoded by nuclear genome. The chloroplast genome encodes only ~100 genes (Timmis *et al.*, 2004). Most of the proteins are synthesized on cytoplasmic ribosomes. Post

translationally these proteins are imported into the organelles from the cytosol and assembled in order to function properly. Here we review the basic steps of protein translocation into both chloroplast and mitochondria by considering both similarities and differences involved. We have tried to summarize the available data from different studies and this might contribute to our understanding of basics of protein import machinery to these organelles.

Protein Import into chloroplast

During evolution, chloroplasts have transferred the majority of their genes to the

nucleus. This gene transfer required the genesis of novel regulatory and targeting mechanisms, which enabled the nuclear encoded proteins an efficient and faithful return to the organelle. Chloroplast contain about 3000 different proteins, 95% of which are encoded in the nucleus, synthesized in the cytosol on cytosolic ribosomes and post translationally translocated/imported into the organelle. More complicated task than protein sorting in mitochondria since chloroplasts contain three distinct internal compartments. A multicomponent translocation machinery located in both the outer and the inner envelope of chloroplasts was identified. Targeting by Presequence is the feature in which N-terminal sequences are significant players. Presequences of known chloroplast proteins are extremely diverse. They can vary in length from 20 to 150 aminoacid residues and appear to have little conservation in their amino acid sequence. Common to all chloroplast precursors, however, is an overall positive charge and an enrichment in the hydroxyl amino acids, serine and threonine.

Vast majority of all known chloroplast proteins use the “classical” Toc-Tic pathway. Hsp70 (heat shock protein of 70 kDa) interact with all kinds of chloroplast precursor proteins the moment they emerge from the ribosome. Their function is to prevent the premature folding of the precursor proteins, because the import machinery admits only unfolded proteins. Guidance complex: Interaction with the guidance complex, increases the import rate of the precursor protein several-fold.

The term Toc-translocon denotes the complete set of proteins involved in the translocation of precursor proteins across the outer membrane of the chloroplast envelope and Toc translocon comprises a core of three proteins Toc159, Toc75 and Toc34. These core proteins build a molecular machine that

is responsible for the recognition of the precursor protein as well as its translocation across the outer membrane. Toc75 is the most prominent protein and it is deeply embedded into the membrane and it can form a beta-barrel anion channel formed by 16 transmembrane β -sheets. Toc75 has a binding site for precursor proteins and acts independently of the Toc receptors Toc34 and Toc159. Toc34 is a preprotein receptor and it contains a single transmembrane domain at the C terminus, whereas the N terminal domain is exposed to the cytosol. Phosphorylation plays an important role in regulation of Toc34 activity and preprotein binding. Phosphorylated preproteins engage an oligomeric guidance complex consisting of a 14-3-3 protein dimer and a cytosolic Hsp70. Enclosed within this complex, preproteins represent highly import-competent substrates. While phosphorylation of the precursor protein is not a prerequisite for translocation, phosphorylated precursor proteins exhibit enhanced binding to Toc34. The presequence is cleaved off by the so-called stromal processing peptidase (SPP). SPP removes the complete presequence in one endoproteolytic cleavage step and simultaneously releases the mature protein. Proteins with N-terminal transit peptides are recognized by a guidance complex that targets them To the Toc complex in the chloroplast outer membrane. The transit peptide binds Toc 159 and Toc 34, which are associated with Hsp70, before being passed to the Toc 75 import pore. Passage through the outer membrane also requires ATP hydrolysis by Hsp 70 in the intermembrane space, and possibly the hydrolysis of GTP by Toc 34. Once through the chloroplast outer membrane, the transit peptide is passed to the Tic complex in the inner membrane. The preprotein is drawn through the Tic complex by the action of an Hsp 100. In the stroma, the transit peptide is

removed by the chloroplast stromal processing peptidase (SPP) and protein interacts with Hsp 70.

Protein import into mitochondria

Most mitochondrial genomes do not encode the proteins required for DNA replication, transcription or translation. The genes that encode proteins required for the replication and expression of mitochondrial DNA are contained in the nucleus. Most of the proteins required for oxidative phosphorylation and citric acid cycle are encoded by nuclear genes. It is also reported that some of these genes were transferred to the nucleus from the original prokaryotic ancestor of mitochondria. Approximately 1000 proteins encoded by nuclear genes (more than 95% of mitochondrial proteins) are synthesized on free cytosolic ribosomes and imported into mitochondria as completed polypeptide chains. Import of proteins is more complicated because of the double-membrane structure of mitochondria. Import of mitochondrial matrix proteins is the best understood aspect of mitochondrial protein sorting. Pre sequences are the amino-terminal sequences with positively charged aminoacids and first characterized by Gottfried Schatz. This 20-35 aminoacids that are removed by proteolytic cleavage following their import in to the organelle.

For the import of small molecule transport proteins into the mitochondrial inner membrane there is multiple-pass transmembrane proteins have internal signal sequences, rather than N-terminal Presequences. The internal signal sequences in association with Hsp90 chaperones interact with the Tom70 receptors, from which the transmembrane protein is transferred to the Tom40 channel. In the intermembrane space, the protein is bound by tiny Tim (mobile) proteins that guide it to

the Tim22 complex in the inner membrane. The tiny Tim proteins transfer the protein to Tim54 and then to Tim22 import pore. Internal stop transfer sequences halt translocation, and the protein is transferred laterally into the inner membrane. For the sorting of proteins containing presequences to different mitochondrial compartments mitochondrial proteins with N-terminal presequences can be imported to the outer membrane, inner membrane, or intermembrane Space. The presequences are recognized by Tom20 receptor and transferred to Tom40 Proteins destined for the outer membrane halt translocation in the Tom40 complex and pass laterally into the membrane. Some proteins destined for intermembrane space are translocated through Tom40 but remain in the intermembrane space rather than interacting with the Tim23 complex. Other proteins are transferred through Tim23 into the mitochondrial matrix. Removal of the presequence within the matrix then exposes a second sorting signal that targets these proteins back into either the inner membrane or the intermembrane space through Oxa 1 translocation pore.

References

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