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Review

Protein import into chloroplasts—How chaperones feature into the game ☆

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ABSTRACT

Chloroplasts originated from an endosymbiotic event, in which an ancestral photosynthetic cyanobacterium was engulfed by a mitochondriate eukaryotic host cell. During evolution, the endosymbiont lost its autonomy by means of a massive transfer of genetic information from the prokaryotic genome to the host nucleus. Consequently, the development of protein import machineries became necessary for the relocation of proteins that are now nuclear-encoded and synthesized in the cytosol but destined for the chloroplast. Organelle biogenesis and maintenance requires a tight coordination of transcription, translation and protein import between the host cell and the organelle. This review focuses on the translocation complexes in the outer and inner envelope membrane with a special emphasis on the role of molecular chaperones. This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

Contents

1. Introduction

Chloroplasts are highly specialized organelles, which perform essential functions such as photosynthesis, nitrogen and amino acid metabolism. Like mitochondria they evolved through an endosymbiotic event from once free living prokaryotic cells [1]. To gain control over its newly enslaved component, the majority of the genes were transferred from the endosymbiont to the host genome, leaving the evolving chloroplast with only about 100 protein encoding genes [2]. Although this process allowed the cell to supervise the functions and biogenesis of the organelle, complex mechanisms had to be developed to transport approx. 3000 proteins into the chloroplast. The import process is additionally challenged by the complex organization of the chloroplast sub-compartments,

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since the organelle is enclosed by two distinct membranes and a third independent membrane system, the thylakoids, which harbor the photosynthetic complexes. This results in three separated soluble compartments: the intermembrane space, the stroma and the thylakoid lumen. The plant cell is faced with several obstacles during the targeting of preproteins: (1) specific targeting to the chloroplast or/and other organelles, such as mitochondria or peroxisomes has to be ensured, (2) transport across the outer and the inner envelope membrane and (3) correct targeting and assembly inside the chloroplast, i.e. stroma, thylakoid and thylakoid lumen. Therefore most chloroplast targeted proteins are synthesized as precursor proteins and equipped with an N-terminal transit sequence, which serves as an entry ticket for the designed organelle and is cleaved after the protein has reached its destination [3]. Once the precursor protein has been guided to the chloroplast, a process involving several cytosolic chaperones, the precursor interacts with receptors on the chloroplast membrane surface and is transported through the membranes in a GTP and ATP dependant manner. Two multi-protein translocon complexes (the TOC and TIC complex) facilitate the transport across the outer (TOC—**t**ranslocon at the **o**uter membrane of **c**hloroplasts) and inner (TIC—**t**ranslocon at the **i**nner membrane of **c**hloroplasts) envelope membranes of most preproteins [4,5]. This import system is up to date the best characterized route, although alternative pathways have been described, especially for proteins of the outer and inner envelope. In a second step proteins designated for the thylakoid membrane or the thylakoid lumen are targeted with the help of specific signal peptides. The mechanisms involved derived from the chloroplasts bacterial ancestor and are beyond the scope of this article, yet a number of excellent reviews are available on that subject [6,7]. Since the biogenesis and function of chloroplasts is a very dynamic and adaptive process, the import mechanism of its constituents also provides a powerful way to act as a regulatory element. Regulation of protein import can occur at several steps, starting with the formation of cytosolic chaperone complexes, the involvement of several isoforms of the Toc subunits and a redox-mediated control at the stage of import through the inner envelope.

In this review we will especially focus on targeting of the proteins to the chloroplast and the process taking place at the envelope membranes as well as the regulation of protein import. The function of cytosolic as well as chloroplast chaperones is especially emphasized.

2. Targeting to the chloroplast

The most simplified way to imagine efficient sorting of proteins to their respective organelles would include targeting peptides with distinct features allowing a clear classification. Yet, such a scheme cannot be applied for the identification of chloroplast transit peptides. Although several programs (e.g. Target P [8]) are able to predict the localization of a nuclear encoded protein with reasonable success, transit peptides of chloroplasts do not show very conserved features [3,9]. Instead of being significantly different in comparison with mitochondrial targeting sequences, they even prove to be quite alike in their features. Both, chloroplast and mitochondrial sequences, show an abundance of hydroxylated amino acids (serine) and very few acidic residues, resulting in an overall positive charge of the signaling peptides [3]. This leaves the vital question, how proteins are really sorted, unanswered up to date. Despite the diversity of sequence motifs Lee and coworkers [10] tentatively defined seven subgroups of transit peptides by hierarchical clustering. When taking a closer look at the secondary structures of mitochondrial and chloroplast transit peptides, some differences become evident. Whereas mitochondrial presequences are capable of forming amphipathic helices [11,12], no secondary structure is formed by chloroplast transit peptides; they even have been proposed to form a perfect random coil [13]. This might play a role in their association with molecular chaperones, such as Hsp70, Hsp90 or 14-3-3 proteins, a topic that will be discussed below in detail.

To add to the complexity, there are a number of proteins which are targeted to more than one organelle, exerting similar functions in both organelles. Three mechanistic possibilities have been described, which allow targeting to both, plastids and mitochondria. The destiny of a precursor can be altered on RNA level, when alternative splicing of the transcript generates different transit signals or different start codons are used for translation of the preprotein leading to different N-terminal sequences. However, some proteins possess ambiguous targeting signals [14–17], raising the question how the distribution between the organelles is monitored and regulated on protein level.

3. Cytosolic components

In the past decade several novel cytosolic components have been assigned to play a role in protein targeting to the chloroplast in addition to the detailed investigation of the translocon complexes. Among these are mainly proteins functioning as chaperones, which associate with the freshly synthesized precursor proteins, thus keeping them in an import competent state and preventing aggregation. Their possible roles in regulation of protein import or discrimination between ambiguous transit signals, however, remain to be established. Most precursor proteins, either chloroplast or mitochondrial targeted, have a potential to bind the heat shock protein Hsp70, which is a highly conserved chaperone, with well described features in regard to its ATP-dependent and co-chaperone mediated assistance in protein folding [18]. Binding of cytosolic Hsp70 to mitochondrial and chloroplast precursor proteins has been shown in several in vitro experiments [19–22]. Since ca. 80% of the chloroplast transit peptides have an Hsp70 binding site [21,23] and Hsp70 was shown to bind to the transit peptides of the small subunit of RubisCo as well as FNR [19,21], binding in the N-terminal region of the precursors is likely. However, binding to the mature part of preproteins has also been observed [24]. Even 97% of the mitochondrial signal peptides contain binding motifs for Hsp70 and they have been shown to play a role in Hsp70 binding *in vitro* [25,26]. Additionally, a Hsp70 bound to the outer envelope membrane, facing the cytosol was identified in spinach, *com*70 [27], which has a potential to interact with precursor proteins as well.

Two further cytosolic components were identified in association with Hsp70s. A 14-3-3 dimer was shown to bind to the transit peptide of the small subunit of RubisCo and other precursors. Binding occurs at a phosphorylated 14-3-3 binding site, which was detected in these precursors [24]. The kinase responsible for the phosphorylation of these precursors could be identified and isolated from *Arabidosis* cytosol preparation. It belongs to a family of three homologous pant specific STY-kinases, containing a serine/threonine as well as a tryrosine phosphorylation domain [28]. The formation of this so-called guidance complex might well have a regulatory or discriminative function, since mitochondrial precursors do not form such complexes. Increased import efficiencies were shown for complexed preproteins in comparison with free precursors [24]. However, targeting was not affected by removal of the phosphorylation sites *in vivo* [29].

Apart from binding of 14-3-3, another major chaperone is involved in guiding loosely folded precursors to the chloroplast. Some preproteins were found to associate with Hsp90 in addition to Hsp70. Like Hsp70, Hsp90 is a well-described chaperone in other prokaryotic and eukaryotic organisms, where it is mainly known to assist the folding of transcription factors and protein kinases with the assistance of several co-chaperones [30]. These functions, however, have so far not been described in plants in great detail. The *Arabidopsis* genome encodes for 7 isoforms of Hsp90, four of which are localized in