Review

New Insights into the Mechanism of Chloroplast Protein Import and Its Integration with Protein Quality Control, Organelle Biogenesis and Development

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Abstract

The translocons at the outer (TOC) and the inner (TIC) envelope membranes of chloroplasts mediate the targeting and import of several thousand nucleus-encoded preproteins that are required for organelle biogenesis and homeostasis. The cytosolic events in preprotein targeting remain largely unknown, although cytoplasmic chaperones have been proposed to facilitate delivery to the TOC complex. Preprotein recognition is mediated by the TOC GTPase receptors Toc159 and Toc34. The receptors constitute a GTP-regulated switch, which initiates membrane translocation via Toc75, a member of the Omp85 (*o*uter *m*embrane *p*rotein *85*)/TpsB (*t*wo-*p*artner *s*ecretion system *B*) family of bacterial, plastid and mitochondrial β-barrel outer membrane proteins. The TOC receptor systems have diversified to recognize distinct sets of preproteins, thereby maximizing the efficiency of targeting in response to changes in gene expression during developmental and physiological events that impact organelle function. The TOC complex interacts with the TIC translocon to allow simultaneous translocation of preproteins across the envelope. Both the two inner membrane complexes, the Tic110 and 1 MDa complexes, have been implicated as constituents of the TIC translocon, and it remains to be determined how they interact to form the TIC channel and assemble the import-associated chaperone network in the stroma that drives import across the envelope membranes. This review will focus on recent developments in our understanding of the mechanisms and diversity of the TOC–TIC systems. Our goal is to incorporate these recent studies with previous work and present updated or revised models for the function of TOC–TIC in protein import.

Introduction

Plastids have evolved an array of protein import and suborganellar targeting systems to facilitate establishment of the structural complexity and functional diversity of this class of organelles [1–4]. The majority of information on protein targeting in plastids has been derived from chloroplasts, the predominant form of plastid found in algae and the green tissues of vascular plants. The biogenesis of chloroplasts during the early stages of plant growth and development involves the proliferation and near-complete proteome remodeling of undifferentiated proplastids or non-photosynthetic etioplasts into photosynthetically competent mature chloroplasts [1,5]. These events give rise to mature chloroplasts composed of three independent membrane systems (outer and inner envelope and thylakoid membranes) and three internal subcompartments (envelope intermembrane space, stroma and thylakoid lumen). As such, the mechanisms and regulation of protein targeting in chloroplasts provide an excellent model for understanding the contributions of targeting pathways to the physiological and developmental changes that control organelle biogenesis and function.

The initial step in the targeting of nucleus-encoded chloroplast proteins is mediated by, at least, four known sorting and import systems at the double membrane of the chloroplast envelope [2,3]. These include a cytosolic sorting system that recognizes chloroplast tail- or signal-anchored integral membrane

proteins and facilitates their insertion into the outer envelope, thereby avoiding mistargeting of these proteins to the endoplasmic reticulum (ER) or mitochondrial outer membrane [6]. A second, largely uncharacterized system mediates the targeting of a set of proteins that lack cleavable targeting signals to the chloroplast [7–9]. A third pathway, which is likely a vestige of early events in endosymbiosis, initially targets proteins to the Sec translocon at the ER and subsequently utilizes delivery of the proteins to the chloroplast via vesicle trafficking through the endomembrane system [10–13]. Analyses from chloroplast proteomics studies suggest that up to several hundred polypeptides could utilize these three targeting pathways [14].

In the fourth pathway, nucleus-encoded chloroplast preproteins are imported from the cytoplasm via sequential interactions between their cleavable, N-terminal transit peptides and the translocons at the outer (TOC) and inner (TIC) envelope membranes [2,3]. This pathway constitutes the major protein import system in plastids, mediating the import of ~3500 polypeptides [15,16]. TOC–TIC serves as the initial gateway for the targeting of the majority of inner membrane, stromal and thylakoid proteins, in addition to a select number of outer membrane proteins. The inner envelope and thylakoid membranes contain their own sorting systems, which function downstream of TOC–TIC and appear to consist largely of pathways conserved and adapted from the cyanobacterium-like ancestor of chloroplasts [17]. The core components of the TOC and TIC systems are present across all plant lineages that evolved from primary endosymbiosis, demonstrating the central role of these import systems in chloroplast biogenesis [3,18,19]. Analyses of the components and the mechanism of TOC–TIC function reveal an interesting hybrid of translocon functions adapted from the cyanobacterium-like endosymbiont and those imposed by the eukaryotic host cell. As a result, comparisons between our understanding of the chloroplast protein import system and bacterial export pathways provide novel insights into the workings of these translocons. Furthermore, the recent characterization of functionally diverse isoforms of TOC components and the developmental control of the import system shed light on how protein import has evolved in land plants to accommodate functional adaptation and specialization of plastids [20–22]. This review will focus on recent developments in our understanding of the composition, mechanism and diversity of TOC–TIC import systems. Our goal is not to be exhaustive but to focus on incorporating these recent findings into existing models of translocon function and explore new models that integrate protein import into the network of gene expression, protein targeting and quality control/turnover controlling plastid biogenesis and development.

Function of the TOC Translocon

Cytosolic events

The majority of chloroplast preproteins appear to be targeted to the chloroplast surface in an unfolded state after they are synthesized on cytoplasmic ribosomes. Preproteins bind directly to the TOC complex via interactions between their intrinsic transit peptides and TOC receptors (Fig. 1c). To date, no specific cytosolic targeting factors analogous to the PTS (*p*eroxisomal *t*argeting *s*ignal) targeting receptors for peroxisomes or to the SRP (*s*ignal *r*ecognition *p*article) targeting system for the ER have been identified in chloroplasts (for review, see Ref. [6]). However, the analysis of preproteins synthesized in *in vitro* translation systems has identified the cytosolic Hsp70 and Hsp90 family members as potential molecular chaperones that facilitate delivery of the unfolded protein to the TOC complex (Fig. 1) [23–26].

Cytosolic Hsp90 also has been shown to interact with both the transit peptide and mature regions of some preproteins and its presence stimulates import *in vitro* [26,27]. Hsp90–preprotein complexes generated in wheat germ extracts also contain the Hsp70/Hsp90 organizing protein (Hop) and the immunophilin FKBP73 (Fig. 1a) [27]. Docking of the chaperone complexes at the chloroplast surface involves Toc64, an integral outer membrane protein (Fig. 1a) [28–30]. Toc64 contains a cytosolic tetratricopeptide repeat (TPR) domain typical of proteins that participate in the formation of Hsp70–Hsp90 chaperone complexes in the cytoplasm, and it is proposed to facilitate transfer of the preproteins from Hsp90 to the TOC complex to facilitate targeting (Fig. 1) [30,31]. The model for Hsp90–Toc64 function is analogous to the role for Hsp90 and the mitochondrial outer membrane TPR protein, Tom70, in the targeting of nucleus-encoded proteins to the Tom translocase of yeast mitochondria [32]. Plants lack a Tom70 homolog, suggesting that Tom70 in animals and fungi evolved after divergence of these organisms [33]. However, many plant species contain a mitochondrial homolog of Toc64, OM64, suggesting that plant mitochondria and plastids might have evolved related but specific pathways from a common ancestral gene to assist cytosolic targeting to endosymbiotic organelles [29,33–36].

Hsp70 in association with a 14-3-3 protein of unknown identity increases the efficiency of import *in vitro* using isolated chloroplasts [25,37]. Together, the Hsp70 and 14-3-3 proteins have been designated the cytosolic guidance complex (Fig. 1b). The transit peptides of several abundant preproteins contain consensus motifs for the binding of 14-3-3 proteins and binding is controlled via reversible phosphorylation of transit peptides *in vitro* [37]. This has led to a model in which reversible phosphorylation regulates the delivery of preproteins to TOC