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Review

Intra-plastid protein trafficking: How plant cells adapted prokaryotic mechanisms to the eukaryotic condition ☆

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ABSTRACT

Protein trafficking and localization in plastids involve a complex interplay between ancient (prokaryotic) and novel (eukaryotic) translocases and targeting machineries. During evolution, ancient systems acquired new functions and novel translocation machineries were developed to facilitate the correct localization of nuclear encoded proteins targeted to the chloroplast. Because of its post-translational nature, targeting and integration of membrane proteins posed the biggest challenge to the organelle to avoid aggregation in the aqueous compartments. Soluble proteins faced a different kind of problem since some had to be transported across three membranes to reach their destination. Early studies suggested that chloroplasts addressed these issues by adapting ancient-prokaryotic machineries and integrating them with novel-eukaryotic systems, a process called 'conservative sorting'. In the last decade, detailed biochemical, genetic, and structural studies have unraveled the mechanisms of protein targeting and localization in chloroplasts, suggesting a highly integrated scheme where ancient and novel systems collaborate at different stages of the process. In this review we focus on the differences and similarities between chloroplast ancestral translocases and their prokaryotic relatives to highlight known modifications that adapted them to the eukaryotic situation. This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

1. Introduction

Chloroplast evolved from an endosymbiotic cyanobacterium. During the process of becoming a chloroplast, the endosymbiont lost most of its ~3000 genes. A large number of these were relocated to the nucleus where they acquired eukaryotic promoters and in most cases targeting peptides for returning the now nucleus encoded and cytosolically synthesized proteins back into the organelle. In addition, because these proteins carry out ancestral functions in topologically comparable compartments, the imported proteins require further trafficking into the different sub-compartments. The cell solved this complex logistical problem by a hierarchical trafficking scheme in which novel translocases in the chloroplast envelope import precursor proteins and deliver them to ancestral protein translocases located in specific chloroplast compartments (Fig. 1). This routing process is known as conservative sorting [1]. Conservative sorting is arguably the most parsimonious solution to the problem as it makes use of pre-existing mechanisms and translocases and requires minimum introduction of novel translocation mechanisms.

Toc (*T*ranslocon at the *o*uter *c*hloroplast envelope) and Tic (*T*ranslocon at the *i*nner *c*hloroplast envelope) are novel translocases located in the chloroplast envelope [2,3]. Toc and Tic import most chloroplast proteins across the envelope into the stroma. Precursor proteins are targeted to Toc/Tic by amino terminal 'transit peptides' that are removed in the stroma following import. Proteins that possess no additional sorting signals remain in the stroma, whereas imported proteins with additional targeting signals are directed to the inner envelope membrane, the thylakoid membrane, or the thylakoid lumen [2]. The ancestral translocases include the thylakoid cpSRP/Alb3 (*chlorop*last *S*ignal *R*ecognition *P*article/Albino3), cpTat (*T*win *a*rginine *t*ranslocation), and cpSec (cpSec1) pathways. All of these systems are represented in extant prokaryotes where they perform analogous functions. A second Sec pathway (cpSec2) has recently been discovered and shown to be largely located in the plastid envelope, although some may be present in the thylakoids [4]. The substrates of cpSec2 have not been definitively identified, but cpSec2 is essential for plastid biogenesis. Taken together, this collection of novel and ancestral translocases appears capable of localizing all of the nuclear encoded proteins. However, adaptations of the ancestral mechanisms have been necessary to address the fact that all localizations of imported proteins in chloroplasts occur post-translationally, whereas the integration of many membrane proteins in bacteria occurs co-translationally [5]. This review aims to summarize the mechanistic features of ancestral

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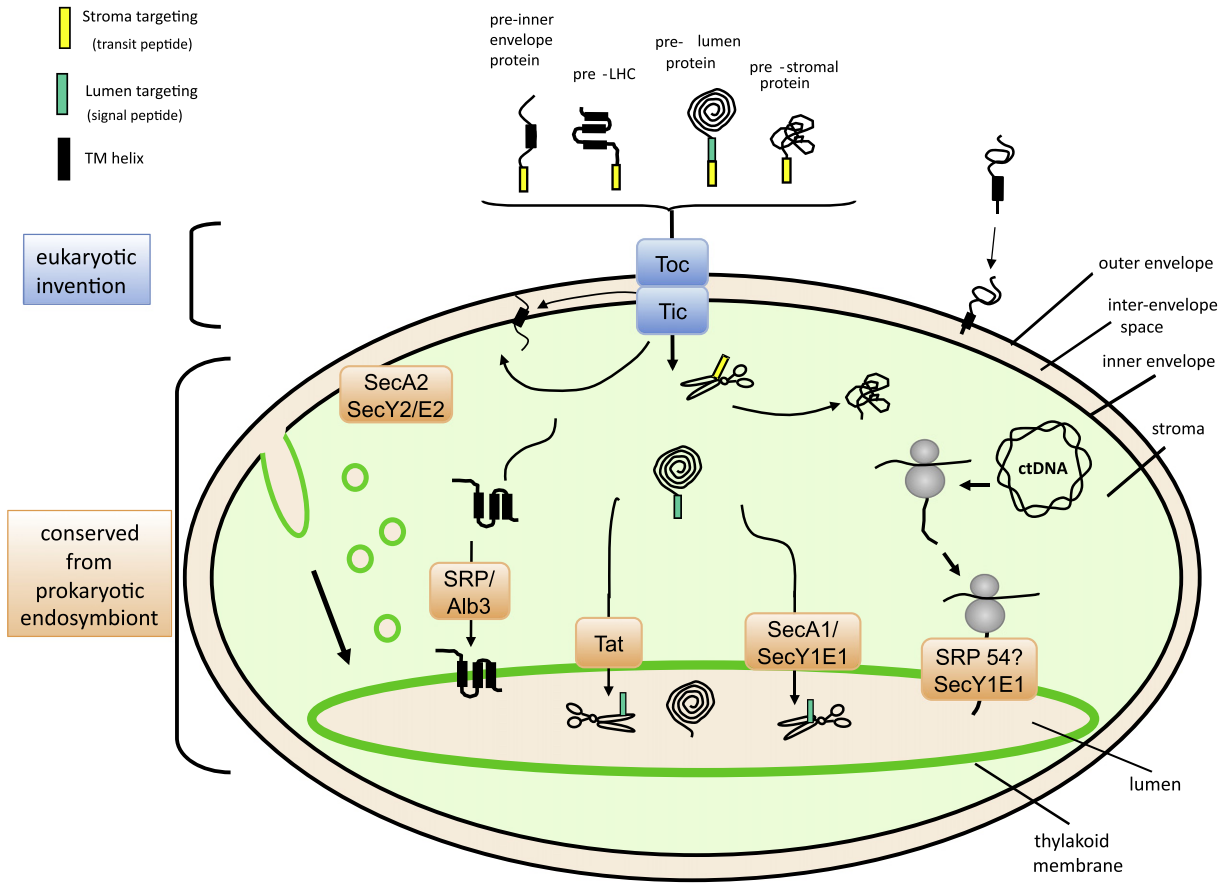

Fig. 1. Trafficking pathways of chloroplast proteins. Most plastid proteins are encoded on nuclear genes and synthesized in the cytosol as precursor proteins with N-terminal transit peptides that govern import through the Toc and Tic translocases into the stroma and are removed by a stromal processing protease. Thylakoid lumen-resident proteins and some thylakoid membrane proteins are targeted by hydrophobic signal peptides that are removed by a lumen-facing signal peptidase following translocation. Multispanning membrane proteins are targeted by uncleaved hydrophobic transmembrane domains (TMD). The ancestral (conserved from the endosymbiont) thylakoid translocases are the cpSRP/Alb3, cpTat, and cpSecA1/cpSecY1E1. A second recently described and divergent Sec translocase, cpSecA2/cpSecY2E2 is located in the plastid envelope. For presentation purposes, the cp prefix is not shown. A relatively small number of plastid proteins are encoded on plastid genes and many of these are co-translationally integrated into the thylakoid membrane and assembled into photosynthetic complexes. A hypothesized, but experimentally supported, membrane flow from the inner envelope membrane to the thylakoids (see Section 3.3) may be involved in thylakoid biogenesis. In this and subsequent figures, translocases conserved from the endosymbiont are colored orange and those invented in eukaryotes or not conserved are colored blue.

translocases, to describe known modifications that adapt them to the eukaryotic situation, and to offer speculative solutions to others.

2. Conservative sorting to the thylakoid membrane and lumen

The proteins localized to thylakoids can be divided into two major classifications according to the types of translocation that they must undergo. Thylakoid lumen resident proteins are globular proteins that must be completely transported across the membrane. Because these are aqueous soluble proteins, crossing the lipid bilayer presents a large energy barrier. Thylakoid membrane integrated proteins fall into several classes including simple, multispanning, and those that have large transported domains, i.e. loops and/or tails.

2.1. Post-translational transport of proteins to the lumen

Proteomic studies of the thylakoid lumen compartment indicate that there are ~80 to 100 lumenal proteins [6,7]. All are encoded in the nucleus and imported into chloroplasts. Lumen resident proteins are transported by either the cpSec1 translocase or the cpTat translocase. Substrate proteins are targeted to these translocases by cleavable hydrophobic signal peptides, with characteristic tripartite amino proximal charged (N) domain, hydrophobic core (H) domain, and cleavage (C) domain, the latter which contains an A-X-A consensus site for the lumen facing signal peptidase, where X designates any amino acid [8]. One major difference between signal peptides for the two pathways is that Tat-directed precursor proteins have an essential twin arginine motif at the intersection of N and H domains [9]. Based primarily on the presence or absence of the twin arginine motif in signal peptides, it is estimated that cpSec1 transports about 50% of the lumenal proteins and cpTat transports the other 50% [6,7]. Targeting specificity for cpSec1 or cpTat is very high both in vitro [9–12] and in vivo [13]. Targeting specificity is determined by the presence/absence of the twin arginine motif, by other more subtle differences in the signal peptide (e.g. hydrophobicity, basic residues in the C domain), and a general incompatibility of cpTat passenger proteins (i.e. the mature domains) with the Sec mechanism (see e.g. [12,14,15] for discussion). This incompatibility is likely related to the fact that at least some cpTat substrates fold tightly in the stroma [16,17].

2.2. cpSec1 transports unfolded proteins through a narrow channel

The cpSec1 system (cpSecA/cpSecYE) was the first ancestral translocase to be identified in chloroplasts [18,19] (Fig. 2A). It is highly homologous to the bacterial SecA/SecYEG system, which operates in a post-translational mode of transport and has been extensively investigated at the mechanistic level [20,21] (Fig. 2B). SecYEG is a transmembrane channel complex that provides the protein-conducting pore. SecA is an