Review

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Shredding the signal: targeting peptide degradation in mitochondria and chloroplasts

Beata Kmiec, Pedro F. Teixeira, and Elzbieta Glaser

Department of Biochemistry and Biophysics, Stockholm University, Arrhenius Laboratories for Natural Sciences, SE-106 91 Stockholm, Sweden

The biogenesis and functionality of mitochondria and chloroplasts depend on the constant turnover of their proteins. The majority of mitochondrial and chloroplastic proteins are imported as precursors via their N-terminal targeting peptides. After import, the targeting peptides are cleaved off and degraded. Recent work has elucidated a pathway involved in the degradation of targeting peptides in mitochondria and chloroplasts, with two proteolytic components: the presequence protease (PreP) and the organellar oligopeptidase (OOP). PreP and OOP are specialized in degrading peptides of different lengths, with the substrate restriction being dictated by the structure of their proteolytic cavities. The importance of the intraorganellar peptide degradation is highlighted by the fact that elimination of both oligopeptidases affects growth and development of *Arabidopsis thaliana*.

Proteolytic events in endosymbiotic organelles

The genome of the model dicot plant *A. thaliana* encodes 723 potential peptidases, constituting circa 2.5% of all genes [1], with the function of most of these peptidases being unknown. According to the recent analysis of the dynamics of the proteome of *A. thaliana* in cell culture, nearly 25% of total protein is degraded, and thus renewed, every day [2]. The average protein degradation rate in the endosymbiotic organelles of developing cells is lower, but the half-life of some organellar proteins can be as low as 30 minutes [2–4]. In addition, protein degradation rates in mitochondria and chloroplasts further increase during senescence, under conditions of environmental or developmental stress, or when the carbon supply is insufficient [4].

Proteolytic events occur also during biogenesis of the mitochondrial and chloroplastic proteins. The involvement of proteolysis in protein biogenesis stems from the fact that the majority of mitochondrial and chloroplastic proteins are synthesized in the cytosol as precursors, carrying cleavable N-terminal targeting signals (see Glossary), termed presequences for mitochondria, transit peptides for chloroplasts, or targeting peptides for both mitochondria and chloroplasts. Such fragments are removed after import into the organelles via a reaction termed processing [5,6]. In this way, both general protein degradation and intraorganellar preprotein biogenesis (through proteolytic processing) result in the generation of free peptides inside endosymbiotic organelles.

Due to their physicochemical properties, accumulation of presequences and transit peptides is potentially harmful. Studies *in vitro* showed that presequence peptides destabilize mitochondrial membranes, dissipating the membrane potential and uncoupling mitochondrial respiration [7–9]. Chloroplastic transit peptides were shown to interact with membrane lipids and cause membrane rupture [10–12]. Additionally, mitochondrial and chloroplastic targeting peptides were shown to inhibit preprotein maturation by inhibition of the processing enzymes [13,14].

Studies in *Caenorhabditis elegans* and *Saccharomyces cerevisiae* have led to the proposal that peptides generated within mitochondria as a result of protein degradation and subsequently exported might influence expression of nuclear genes encoding mitochondrial proteins, thus trigger retrograde signaling [15,16].

Glossary

Presequence: an N-terminal peptide of a precursor protein that directs the protein to mitochondria and is proteolytically cleaved off in the mitochondrial matrix. Presequences might be bipartite, consisting of an N-terminal part directing protein to mitochondria followed by an additional cleavable peptide directing the protein to a specific intra-mitochondrial compartment (e.g., intermembrane space or inner membrane).

Targeting peptide: a cleavable N-terminal peptide of a precursor protein that directs the protein to mitochondria or chloroplasts. Targeting peptide is a more general term to describe peptides for organellar recognition and comprises both presequences and transit peptides.

Targeting signal: a general term describing a sequence that directs proteins either to an organelle (e.g., mitochondria or chloroplasts) or to an intra-organellar compartment. Targeting signals may or may not be proteolytically removed. In contrast to the mitochondrial or chloroplastic proteins containing cleavable targeting peptides, some organellar proteins contain uncleavable signals (e.g., mitochondrial membrane proteins usually contain an internally localized hydrophobic targeting signal; intermembrane space proteins contain a characteristic internal Cys motif functioning as a targeting signal).

Transit peptide: an N-terminal peptide of a precursor protein that directs the protein to chloroplasts and is proteolytically cleaved off in the chloroplastic stroma. Transit peptides might be bipartite, consisting of an N-terminal part directing protein to chloroplasts followed by an additional cleavable peptide directing the protein to a specific compartment within chloroplasts (e.g., thylakoid membrane or lumen).

Corresponding authors: Kmiec, B. (beata.kmiec@dbb.su.se); Glaser, E. (e_glaser@dbb.su.se).

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Box 1. Outstanding questions

- What is the molecular basis for the observed phenotype in plants lacking both PreP and OOP oligopeptidases? It is possible that the observed phenotype results from toxic effects of accumulating targeting peptides or from a disturbance of the general protein degradation pathway. *prep1 prep2 oop* triple knockout plants provide a good model to study consequences of lack of peptide degradation *in vivo*.
- How is organellar import of PreP and OOP regulated? Both PreP and OOP are encoded by a single gene, expressed with an ambiguous targeting peptide dually recognizing receptors on mitochondria and chloroplasts. The volume and envelope surface of chloroplasts are much larger than those of mitochondria, which raises the question how the proper organellar distribution of the peptidases is achieved.
- Are there systems for peptide export in plant endosymbiotic organelles? Analyses in *Saccharomyces cerevisiae* showed that peptides generated in mitochondria can be exported from the organelles by the action of the ABC-type transporter Mdl1, even though the deletion of Mdl1 in yeast does not result in any phenotype. Despite the existence of possible sequence homologues of Mdl1 in *Arabidopsis thaliana*, such systems for peptide export have not been characterized in plant mitochondria or chloroplasts.
- Does a similar complementary system for peptide degradation operate also in humans? Peptidases of M16 and M3 families are evolutionarily conserved, from bacteria to mammals. Sequence homologues of both PreP and OOP have been identified also in humans [69,79]. The only human homolog of PreP, hPreP, has been directly shown to degrade mitochondrial presequences as well as Aβ peptides, and its lower activity has been linked to the progression of AD [69,71,80]. The mitochondrial function of the human homolog of OOP remains to be characterized as well as the possibility of PreP and OOP cooperating in the degradation of human mitochondrial presequences and Aβ peptides.

Due to their potential toxicity and influence on cell metabolism, the turnover of peptides generated in mitochondria and chloroplasts is likely to be regulated. This regulation could be achieved by either the export of peptides to the cytosol or local degradation within the organellar compartments. Studies in yeast suggested that targeting peptides could be potentially exported from the organelles through dedicated ABC-type transporters [17]. However, such putative systems involved in peptide export have not yet been identified in plant mitochondria or chloroplasts (Box 1). The only characterized strategy for mitochondrial and chloroplastic targeting of peptide removal in plants is proteolytic degradation. In this review, we will describe the processes leading to the generation of peptides in mitochondria and chloroplasts as well as recent developments in understanding their degradation by organellar oligopeptidases.

Preprotein processing – generation of free targeting peptides

Mitochondrial biogenesis starts during the early stages of plant germination with mitochondria of a typical morphology observed already after 60 hours (48 hours of dark stratification followed by 12 hours in continuous light). By contrast, biogenesis of chloroplasts requires longer exposure to light [18]. The majority (>95%) of mitochondrial and chloroplastic proteins are synthesized in the cytosol and post-translationally imported. Proper biogenesis and function of endosymbiotic organelles require import of approximately 1400 proteins to mitochondria and between 1500 and 2300 proteins to chloroplasts [6,19–22].

To ensure proper targeting and import, most of the mitochondrial and chloroplastic proteins (70% and 85%, respectively) are synthesized in the cytosol as preproteins containing cleavable targeting signals. These signals are attached to the mature part of the protein as N-terminal extensions and recognized by receptor proteins localized in the organellar outer membranes [6,23,24]. After binding to the outer membrane receptors, preproteins destined for the mitochondrial inner membrane and matrix are translocated inside mitochondria through the translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) complexes. Chloroplastic preproteins, destined to reside in the stroma or thylakoids, are translocated across the outer and the inner chloroplast envelope by analogous import machinery – the translocon at the outer chloroplast envelope (TOC) and the translocon at the inner chloroplast envelope (TIC). Chloroplastic proteins destined for thylakoid compartments possess an additional signal, the thylakoid transfer domain. This simplified view is true for most mitochondrial and chloroplastic proteins. Various aspects of the organellar protein import have been extensively reviewed elsewhere [25–32].

The import fidelity of precursor proteins from the cytosol to their specific location within the mitochondria and/or the chloroplasts is determined by the distinct features of the mitochondrial and the chloroplastic targeting peptides. The mitochondrial presequences differ from the chloroplastic transit peptides in their average length, distribution of amino acids, and propensity to form secondary structures [5,10,33–35]. Based on the analysis of the N termini of mature proteins it was estimated that the mitochondrial presequences are 11–109 amino acids (aa) long [5,36], with most of the peptides ranging between 21 and 40 aa in *A. thaliana* [5]. By contrast, the chloroplastic transit peptides range from 26 to 146 aa with the majority possessing between 41 and 70 aa [6]. Despite sharing similarities in the overall amino acid composition (the most abundant residues in both types of targeting peptides are serine, leucine, alanine, and arginine), there are subtle differences in the N-terminal portion of the targeting peptides, with arginine overrepresented in the presequences and serine and proline overrepresented in transit peptides [5,37]. Presequences have a propensity to form amphiphilic α-helices [5,34,35], whereas transit peptides are mostly unstructured, but can also adopt α-helical conformation in membrane mimetic environments [10,33].

After reaching the mitochondrial matrix or the chloroplastic stroma, preproteins undergo proteolytic processing, during which presequences and transit peptides are cleaved off. This reaction is performed by specific processing peptidases [38]. Most precursors destined for the mitochondrial matrix are processed by the general mitochondrial processing peptidase (MPP), which in plants is integrated into the cytochrome bc_1 complex of the respiratory chain [39,40]. MPP is highly expressed during early stages of *A. thaliana* development, characterized by an intensive mitochondrial biogenesis and import [18]. Studies in yeast confirm that MPP expression is essential for the proper biogenesis of mitochondria [41]. MPP processes