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Review

The plant mitochondrial protein import apparatus — The differences make it interesting☆

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ABSTRACT

Background: Mitochondria play essential roles in the life and death of almost all eukaryotic cells, ranging from single-celled to multi-cellular organisms that display tissue and developmental differentiation. As mitochondria only arose once in evolution, much can be learned from studying single celled model systems such as yeast and applying this knowledge to other organisms. However, two billion years of evolution have also resulted in substantial divergence in mitochondrial function between eukaryotic organisms.
Scope of Review: Here we review our current understanding of the mechanisms of mitochondrial protein import between plants and yeast (*Saccharomyces cerevisiae*) and identify a high level of conservation for the essential subunits of plant mitochondrial import apparatus. Furthermore, we investigate examples whereby divergence and acquisition of functions have arisen and highlight the emerging examples of interactions between the import apparatus and components of the respiratory chain.
Major conclusions: After more than three decades of research into the components and mechanisms of mitochondrial protein import of plants and yeast, the differences between these systems are examined. Specifically, expansions of the small gene families that encode the mitochondrial protein import apparatus in plants are detailed, and their essential role in seed viability is revealed.
General significance: These findings point to the essential role of the inner mitochondrial protein translocases in Arabidopsis, establishing their necessity for seed viability and the crucial role of mitochondrial biogenesis during germination. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

Mitochondria are membrane bound organelles that play essential roles in metabolism, energy production and biosynthesis of a variety of compounds in almost all eukaryotic cells. They are endosymbiotic in origin, and over time the majority of genes in the endosymbiont were lost or transferred to the host nucleus [1]. Thus, the majority of the 1000+ proteins located in mitochondria are encoded by nuclear genes, translated in the cytosol and imported into mitochondria [2]. *Saccharomyces cerevisiae* (yeast) has long been established as the pre-eminent model for the study of mitochondrial protein import [3]. However, many of the components involved in mitochondrial protein import are also well conserved across different species and whilst they have not been characterized in plants to the extent they have been in yeast, the presence of orthologous genes and complexes is well established across plants, animals, fungi [4–6] and even protists to a slightly lesser extent [7,8]. Fig. 1 summarizes the various pathways and complexes involved in protein import in plant mitochondria.

A multi-subunit protein complex on the mitochondrial outer membrane, termed the Translocase of the Outer Membrane (TOM), recognizes mitochondrial precursor proteins and passes them to one of two inner membrane multi-subunit protein complexes, termed the Translocases of the Inner Membrane (TIM) (Fig. 1). One of these is TIM17:23, which is responsible for the import of proteins via the general import pathway, i.e. for proteins that contain N-terminal targeting signals (Fig. 1). Alternatively, TIM22 is responsible for the import of proteins via the carrier import pathway, which is specific for the import of inner membrane proteins containing internal mitochondrial targeting signals (Fig. 1). Along with the Sorting and Assembly Machinery (SAM) complex on the outer membrane and the Mitochondrial Intermembrane space Assembly (MIA) in the intermembrane space,

Abbreviations: TOM, Translocase of the Outer Mitochondrial membrane; OM, Outer Membrane; SAM, Sorting and assembly machinery; TIM, Translocase of the Inner Mitochondrial membrane MIA, Mitochondrial Inter membrane space import and Assembly; ERV, Essential for Respiration and Vegetative growth; MPP, Mitochondrial Processing Peptidase; PreP, Presequence Protease

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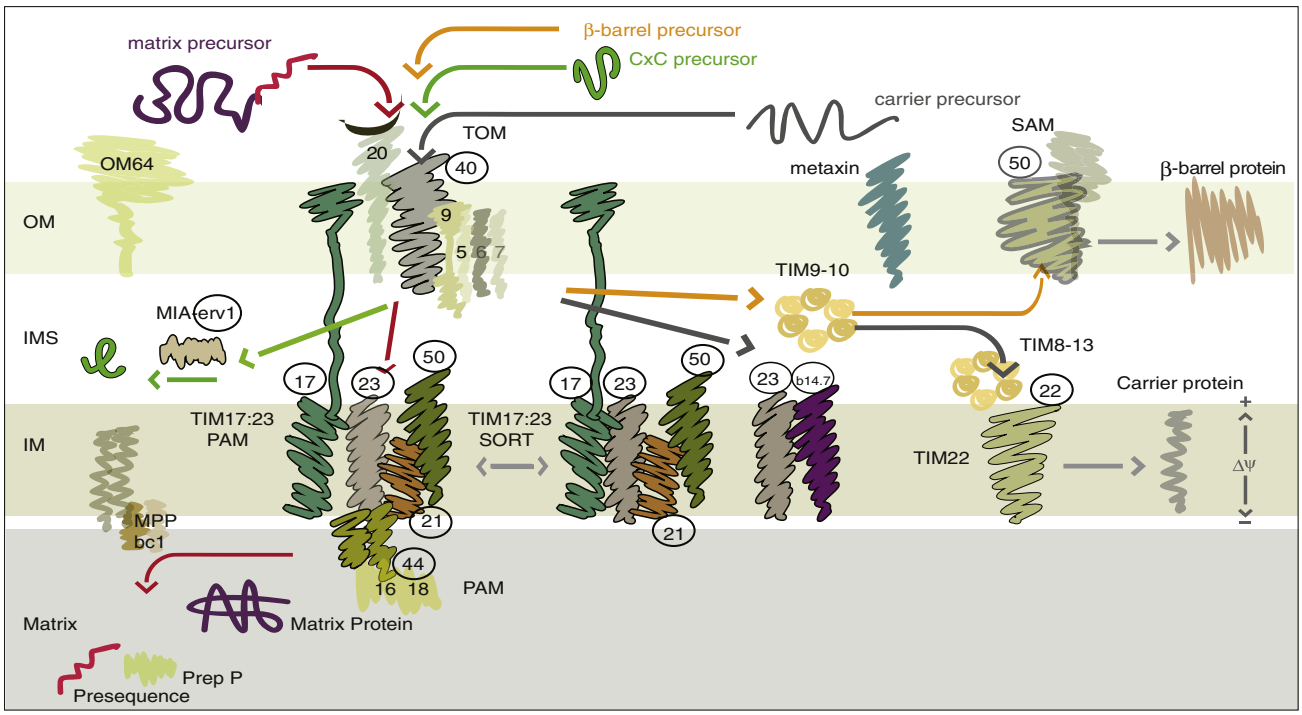

Fig. 1. Diagrammatic representation of protein import pathways and components in plant mitochondria. Targeting signals on cytosolically located precursor proteins determine the pathway of import. All precursors (containing a cleavable presequence, Twin CxC, β-barrel or carrier type proteins) pass through the Translocase of the Outer Membrane (TOM). The pathways diverge in the InterMembrane Space (IMS). Insertion of β-barrel proteins into the outer membrane is carried out via the Sorting and Assembly Machinery (SAM). Carrier import pathway proteins translocate into the inner membrane via small soluble Tim proteins through the TIM22 channel located in the inner membrane. Twin CxC proteins located within the IMS, are imported via the MIA-Erv1 pathway. For the majority of mitochondrial proteins that contain a cleavable presequence, translocation is carried out through the TIM17:23 complex with the Presequence Assisted Motor (PAM) complex or inserted directly into the inner membrane. The TIM17:23 complex can exist as 2 forms, PAM and SORT. In addition Tim23 interacts with the Complex I subunit B14.7. Following translocation, the presequence is removed via Mitochondrial Processing Peptidase (MPP), integrated into the cytochrome bc1 complex. The presequence is further processed via a matrix located Presequence Peptidase (PrepP). The essential subunits required for seed viability are circled. A membrane potential is required for translocation across the inner membrane (ΔΨ). Abbreviations: TOM = Translocase of the Outer Membrane, TIM = Translocase of the Inner membrane, IMS = Intermembrane Space, SAM = Sorting and Assembly Machinery, PrepP = Presequence Peptidase, MPP = Mitochondrial Processing Peptidase. The subunits known to be essential for plant viability are bolded. SAM50 is bolded in grey as no experimental verification of it essential function has been reported in plants.

these complexes are responsible for the import of the majority of proteins into the mitochondria [3,9]. In the last few years, studies on the mechanisms of protein import into mitochondria have revealed interactions between the protein import complexes with other multi-subunit protein complexes. Many of the studies revealing these interactions have been carried out in yeast [10,11]. Additionally, there have been emerging reports of interactions between the TIM17:23 complex and respiratory chain in both yeast and plants, although the biological implications of these interactions are not yet known [12,13].

Whilst most mitochondrial import components are highly conserved between yeast and plants, there are several areas of divergence that have arisen. These include the presence of plant specific mitochondrial import components, as well as the expansion of the gene families encoding these components, resulting in sub-functionalisation and/or neo-functionalisation. Studies characterizing the function of mitochondrial import components in yeast have revealed that several are essential for viability [14]. Interestingly, studies in plants have revealed a requirement for mitochondrial import components very early during germination in Arabidopsis (*Arabidopsis thaliana* — At) and rice (*Oryza Sativa*), when organelle biogenesis is actively occurring [15–17]. This early requirement is also supported by the findings that knocking-out certain import components, such as AtTIM50, results in embryo lethality [18]. To date, over 400 genes have been defined as essential in Arabidopsis [18]. In this review, the plant mitochondrial import components are explored and several inner membrane components are shown to be essential, whereby knocking out these genes results in an embryo lethal phenotype in Arabidopsis.

2. Outer membrane

The recognition of mitochondrial preproteins and the commencement of translocation occur via protein complexes on the outer membrane, namely the TOM complex and the SAM complex. Characterization of the mitochondrial protein import machinery on the outer membrane reveals that whilst the overall process is conserved in both yeast and plants, there are important mechanistic differences [6].

2.1. The TOM complex

The TOM complex consists of the cytosolic facing receptor subunits Tom20 and Tom70, the 'convergent' receptor Tom22 and the pore forming Tom40 channel. Preprotein recognition generally occurs via the N-terminal presequence, present on ~70% of mitochondrial proteins that ranges from 6 to 90 amino acids [19]. Tom20 exhibits substrate specificity with N-terminal presequences containing proteins whilst Tom70 binds proteins with internal non-cleavable targeting signals [20,21]. Tom40, a β-barrel protein, constitutes the translocation pore for almost all mitochondrial proteins and was the first mitochondrial membrane protein shown to be essential for yeast viability. Similarly, in Arabidopsis, T-DNA insertional knock-out lines for At*Tom40-1*, the highest expressed isoform [22] are not viable, suggesting that whilst there are two isoforms, At*Tom40-2* cannot compensate for the loss of At*Tom40-1*.