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Review

Unique components of the plant mitochondrial protein import apparatus ☆

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ABSTRACT

The basic mitochondrial protein import apparatus was established in the earliest eukaryotes. Over the subsequent course of evolution and the divergence of the plant, animal and fungal lineages, this basic import apparatus has been modified and expanded in order to meet the specific needs of protein import in each kingdom. In the plant kingdom, the arrival of the plastid complicated the process of protein trafficking and is thought to have given rise to the evolution of a number of unique components that allow specific and efficient targeting of mitochondrial proteins from their site of synthesis in the cytosol, to their final location in the organelle. This includes the evolution of two unique outer membrane import receptors, plant Translocase of outer membrane 20 kDa subunit (TOM20) and Outer membrane protein of 64 kDa (OM64), the loss of a receptor domain from an ancestral import component, Translocase of outer membrane 22 kDa subunit (TOM22), evolution of unique features in the disulfide relay system of the inter membrane space, and the addition of an extra membrane spanning domain to another ancestral component of the inner membrane, Translocase of inner membrane 17 kDa subunit (TIM17). Notably, many of these components are encoded by multi-gene families and exhibit differential subcellular localisation and functional specialisation. This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

1. Introduction

All mitochondria are thought to have descended from a single endosymbiotic event ~2 billion years ago [1]. Over the course of evolution, much of the genetic material from the endosymbiont was transferred to the nuclear genome [2], thereby creating a requirement for the transport and import of these proteins back to their site of function in the organelle. The mechanisms and many of the core components involved in this process are likely to have been established in the earliest eukaryotes [3]. This is evidenced by the conservation of many of the central pore forming components in animals, plants and fungi; including the translocase of the outer membrane (TOM) pore TOM40 [4], the sorting and assembly machinery (SAM) core subunit SAM50 [5] and the translocase of the inner membrane (TIM) complexes TIM17:23 and TIM22, all of which are conserved across the eukaryotic kingdoms [3] (Fig. 1). However, in the estimated 2 billion years following the common origins of these proteins, the conserved core components have diversified and additional, lineage specific components have evolved to fulfil the specific requirements of mitochondrial protein import in the varying conditions and cytosolic environments across these three kingdoms [6].

The arrival of the plastid in the plant lineage, as long as 1.5 billion years ago [7], is thought to have posed a new challenge to the previously established mitochondrial protein import apparatus [8]. Similar to the mitochondria, much of the plastid genetic material was subsequently transferred to the nucleus [8] and this introduced complications in the process of protein trafficking [8]. Nuclear encoded mitochondrial and plastid proteins are commonly targeted by the presence of an N-terminal, cleavable presequence. These presequences share several common features such as a region enriched in basic amino acids and a C-terminal domain predicted to form amphipathic α-helices [9,10]. It has been demonstrated that such signals are similar enough that the inclusion of a plastid targeting sequence from plants is capable of efficiently targeting proteins to mitochondria in yeast [11]. The selective pressure to maintain the specificity and efficiency of protein trafficking in the face of this additional complexity is thought to have led to the evolution of a number of unique features in the plant mitochondrial protein import apparatus that form the subject of this review. The availability of whole genome sequence data has greatly facilitated comparative analysis of the mitochondrial protein import apparatus within plants, as well as in comparison to other model organisms such as yeast [12,13]. Whilst this review principally deals with the mitochondrial import apparatus of *Arabidopsis*, other species are also addressed where relevant.

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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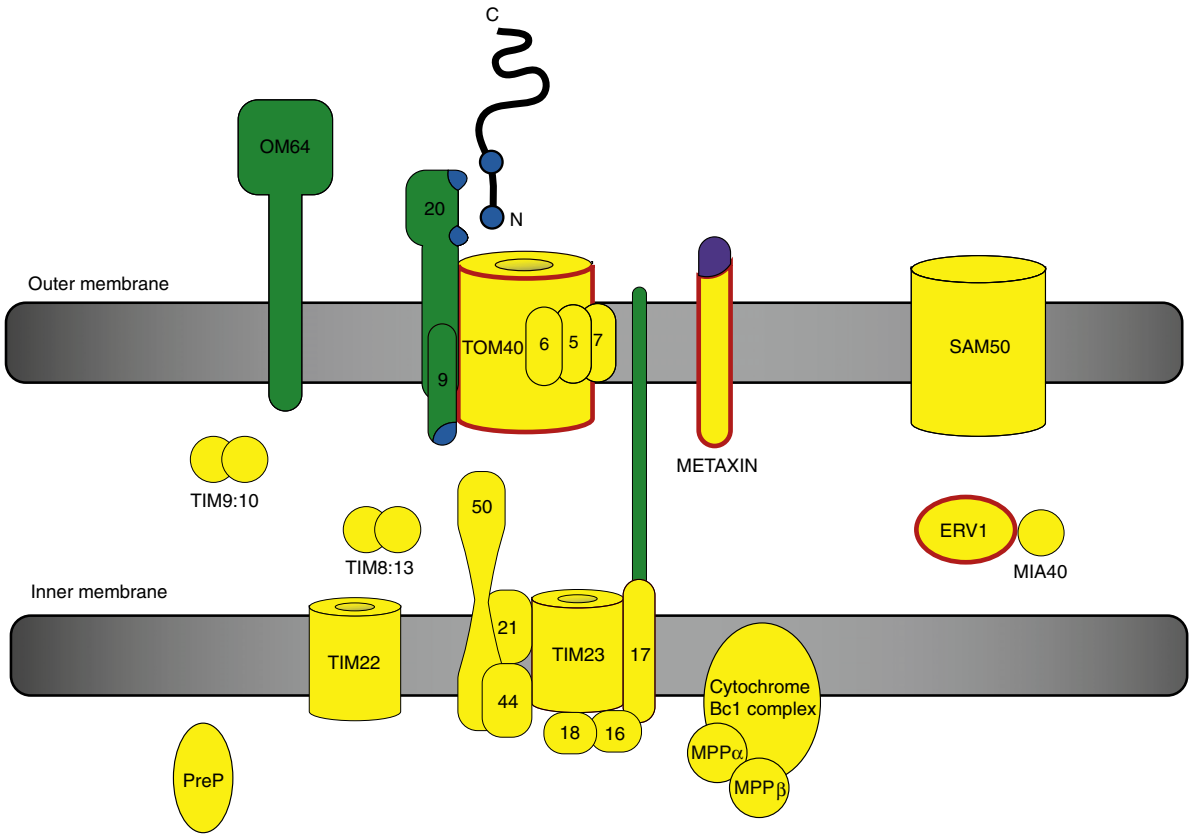

Fig. 1. The mitochondrial protein import apparatus of plants. Conserved components found in members of all of the eukaryotic lineages are shown in yellow. Plant specific components are shown in green and include — TOM20, TOM9, OM64 and the C-terminal regions of TIM17. Components which have been shown to be essential for normal plant development are outlined in red and presequence interacting domains are shown in blue. 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.

2. Outer membrane

Specific recognition of mitochondrial preproteins and the initiation of their import are processes that have been intensively studied in *Saccharomyces cerevisiae* (yeast), *Neurospora crassa* and *Rattus norvegicus* (rat) [14–16]. In the general import pathway, mitochondrial proteins are targeted by the presence of an N-terminal presequence, which is specifically recognised by receptors on the outer membrane and translocated through the TOM complex. The TOM complex (Fig. 1) is the principal outer membrane import complex and with few exceptions [17] all mitochondrial proteins are thought to pass through the pore forming TOM40 subunit [18,19]. In yeast, this complex is composed of TOM40, the secondary receptor and central organiser, TOM22, the small TOMs, TOM5, 6 and 7, which regulate the formation and function of the complex, and the two cytosolic facing receptor subunits TOM20 and TOM70 [18,20]. Studies on the various presequence interacting components in yeast have led to a model in which precursors are recognised and passed through the TOM40 pore to the inner membrane or inter membrane space by a system of increasing affinity. Each subsequent import component in the binding chain interacts with the presequence with greater affinity than the last, allowing the transport of mitochondrial proteins across the outer membrane in an ATP independent manner [21,22]. In yeast, mutant strains lacking either of the two principle import receptors TOM20 or TOM70 are viable but are seen to grow slowly and import nuclear encoded proteins at rates considerably lower than wild type, indicating that these components are important but not crucial for viability [23]. Yeast TOM20 is anchored to the outer mitochondrial membrane by an N-terminal α-helically anchored protein with a large cytoplasmic domain consisting of a tetratricopeptide (TPR) repeat motif capable of directly interacting with nuclear encoded mitochondrial proteins *via* N-terminal targeting signals [16,24]. In yeast, TOM70, like TOM20, functions as an import receptor, though it does so through interaction with the cytosolic chaperones HSP90 and HSP70 [25]. TOM70 contains a total of seven TPR domains divided into two functional groups. The first three from the N-terminal form a dicarboxylate clamp, which interacts with the cytosolic chaperones, passing the inbound mitochondrial protein to the core domain of TOM70, which recognises targeting signals internal to the preprotein [26]. Multiple TOM70 dimers are then recruited to the complex and the protein is passed through the import pore in an ATP dependent manner [25].

Interestingly, whilst orthologues to TOM20 or TOM70 of animals and fungi are absent from the plant mitochondrial outer membrane, proteins of similar size and function are found (see below), suggesting that TOM20 and TOM70 in yeast and animals evolved subsequent to the divergence of the eukaryotic lineages [27]. Whilst this may explain the absence of these receptors in plants, the core, conserved components of the outer membrane import apparatus similarly demonstrate divergent structures in plants. Most notably, the cytosolic receptor domain of yeast and animal TOM22 [28] has been lost from the plant TOM22 protein, suggesting that a fundamentally different presequence binding chain is operational in plants [4]. Similarly, amino acid residues in yeast TOM40 that have been shown to be important for the transfer of preproteins between the TOM and the TIM17:23 complexes [29] are absent from *Arabidopsis* TOM40. It has been proposed that these differences in the outer membrane protein complexes of plants occurred as a result of both the independent origins of the components involved, and the greater need for specificity in the recognition of *bona fide* mitochondrial proteins in the presence of plastid proteins [8].