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Review

The preprotein translocase of the outer mitochondrial membrane: receptors and a general import pore

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Abstract. Cytosol-synthesized preproteins destined for the mitochondria are transported across the outer membrane by the translocase of the mitochondrial outer membrane (TOM complex). This dynamic transport machinery can be divided into receptors that recognize preprotein targeting signals and components of the general import pore complex that mediate preprotein transport across the outer membrane. This review focuses on recent studies dealing with the central questions regarding the pore-forming subunits, and architecture and gating of the translocation channel of the outer membrane.

Key words. Mitochondria; translocation; protein import; protein interaction; channel.

Introduction

Most mitochondrial proteins are synthesized as precursors in the cytosol. Their subsequent import into mitochondria is mediated by translocation machineries in the outer membrane (translocase of the outer membrane; TOM complex) and in the inner membrane (translocase of the inner membrane; TIM complex) [1–3]. The components of the TOM complex can be divided into the receptor subunits Tom20, Tom70, and Tom22, and subunits of the general import pore (GIP) complex comprised of Tom40, Tom22, Tom7, Tom6, and Tom5 [the number indicates the apparent molecular weight in kilodaltons; for the nomenclature see ref. 2]. The Tom receptors recognize and bind to preproteins either containing N-terminal, positively charged presequences or those harboring internal targeting information. Following recognition by Tom receptors, the preprotein is translocated across the outer membrane at the GIP.

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While the import route across the outer membrane is common for most preproteins, the preprotein is faced with at least three different pathways when it reaches the intermembrane space. Presequence-carrying preproteins destined for the mitochondrial matrix are translocated across the inner membrane via the TIM23 complex consisting of Tim23, Tim17, and associated Tim44 as well as the matrix-located heat shock protein 70 (mtHsp70). Many preproteins destined for the inner membrane, such as those of the metabolite carrier family, contain internal targeting sequences and utilize a different translocase, the TIM22 complex. The known components of this translocase are Tim54, Tim22, and Tim12 [4–7]. On their way across the intermembrane space (IMS), the carrier preproteins associate with a number of small, soluble Tim proteins that may mediate a chaperone-like function by preventing aggregation of the hydrophobic preprotein in the aqueous milieu (fig. 1) [6–8]. Some preproteins destined for the IMS do not engage with TIM components and hence only utilize the TOM machinery for their import [9, 10].

This review focuses on recent studies that have contributed to a more detailed understanding of the function of the translocation machinery of the mitochondrial outer membrane. Readers interested in the process of preprotein translocation into and across the inner membrane are referred to specialized TIM reviews [11, 12].

The import receptors of the outer membrane

Purification of the TOM complex from *Neurospora crassa* using Tom22 containing a polyhistidine tag revealed that all receptors can be coeluted with the components of the GIP [13]. However, other studies employing *Saccharomyces cerevisiae* mitochondria suggest that the association of TOM components with one another may be more dynamic [14]. Indeed, many different direct interactions between members of the TOM machinery have been reported (table 1). A number of such interactions may be mediated by tetratrico peptide repeat (TPR) motifs which are found in a number of TOM components [1]. These motifs are degenerated 34-residue sequences that facilitate protein-protein interactions by forming hole-and-knob-like structures [15].

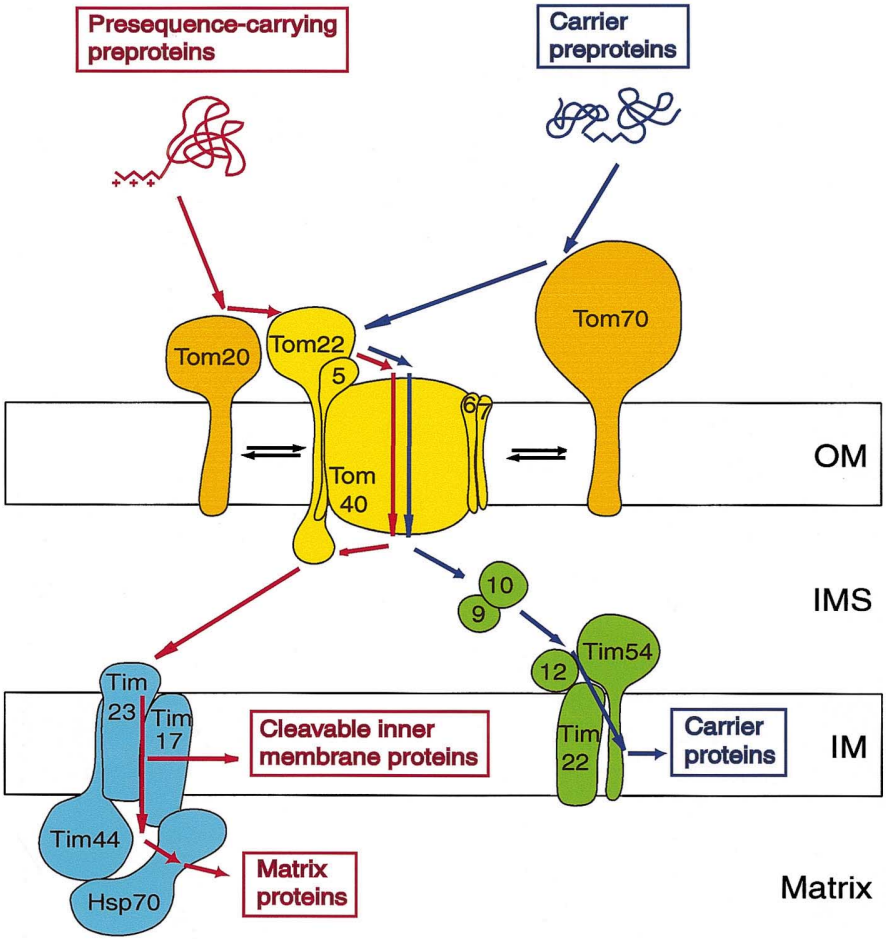

Figure 1. Model of the two major targeting routes of preproteins into mitochondria. Outer membrane (OM): presequence-carrying preproteins bind preferentially to the recepor Tom20 while carrier preproteins preferentially bind to Tom70. Both classes of preproteins are then transferred to the translocation pore (Tom40) via interactions with Tom22 and Tom5. Inner membrane (IM): following translocation across the outer membrane, presequence-carrying preproteins may bind to trans-binding sites before insertion into the TIM23 complex (Tim23, Tim17 associated with Tim44, and mtHsp70). Preproteins are then sorted into the matrix or into the inner membrane. Some preproteins destined for the inner membrane and lacking N-terminal presequences (e.g., carrier preproteins) cross the intermembrane space (IMS) by interacting with soluble Tim complexes (Tim9/Tim10) that transfer them to the TIM22 complex (Tim22, Tim54, Tim12).

Table 1. Reported direct interactions between TOM components and with preproteins.

Tom70	Tom40	Tom37	Tom22	Tom20	Tom7	Tom6	Tom5	
Binding studies [20, 48, 58] Cross-linking [18, 31, 47, 55]	Cross-linking [31, 35, 47, 56, 60] Electrophys. [39]	-	Binding studies [20, 23, 48, 59] Cross-linking [18, 31, 47, 60]	Binding studies [20, 48] Cross-linking [18, 24, 31, 47, 61]	-	-	Cross-linking [22, 55]	**Preprotein**
Cross-linking [55]	-	Immunop. [30]	Binding studies [40] Immunop. [40]	Cross-linking [57] Genetic [57] Immunop. [57]	-	-	-	**Tom70**
	Cross-linking [40, 56]	-	Binding studies [40] Immunop. [40] Native PAGE [14, 40]	-	Native PAGE [14]	Cross-linking [56] Genetic [42] Immunop. [43] Native PAGE [14]	Immunop. [22] Native PAGE [14]	**Tom40**
		-	-	-	-	-	-	**Tom37**
			-	Binding studies [40] Cross-linking [19] Immunop. [40]	-	-	-	**Tom22**
				-	-	-	-	**Tom20**
					-	-	-	**Tom7**
						-	-	**Tom6**
							-	**Tom5**

Immunop., immunoprecipitation; Binding studies, studies using soluble domains; Genetic, genetic analysis; Electrophys., electrophysiological studies.

Tom20/Tom22

Tom20 and Tom22 chiefly recognize and bind typical mitochondrial preproteins carrying N-terminal presequences that can form positively charged amphipathic structures. Tom20 has an N-terminal membrane anchor and a C-terminal cytosolic domain, whereas Tom22 consists of an N-terminal cytosolic domain that is separated from a negatively charged IMS located C-terminal domain by a single membrane anchor [16–19]. The specific binding of presequences to these receptors was shown using purified cytosolic domains of Tom20 and Tom22. While the interaction of preproteins with Tom22 seems to be more ionic and located towards the C-terminal end of the presequence, Tom20 associates preferentially with the N-terminal end in a more hydrophobic manner [20]. These results support the concept that Tom20 and Tom22 can bind amphipathic presequence helices in a coincident and co-operative fashion. At a second receptor level, Tom22 functions as a common final receptor for both classes of preproteins by transferring them from either Tom70 or Tom20 into the import pore with the aid of Tom5 [21, 22]. On the IMS side of the GIP, Tom22 further provides a trans-binding site for incoming preproteins. This IMS-located domain is rich in negative charges and most likely plays a role as an ionic binding site for presequence-carrying preproteins as they are translocated across the outer membrane (see below) [23, 24].

Tom70 and cofactors

The topology of Tom70 is similar to that of Tom20, however, the receptor domain of Tom70 is larger which most likely enables this receptor to conduct multiple interactions with preproteins [25, 26]. This feature could explain the preference of Tom70 for preproteins containing (several) internal targeting sequences. Binding studies using the purified cytosolic domain of Tom70 indeed showed that several internal parts of a preprotein are recognized [27].

Tom70 further interacts with preproteins that utilize the aid of cytosolic factors to guide them to the mitochondria. One such factor is the mitochondrial import stimulation factor (MSF) which, with the hydrolysis of ATP, directs and transfers preproteins to members of the TOM machinery [28]. Cytosolic hsp70 was also shown to guide preproteins to the mitochondria; however, it is not specific for this organelle and can also deliver proteins to other cellular compartments [28, 29]. The role of these cytosolic factors may be to prevent aggre-

gation or irreversible folding of the preproteins on their way to the mitochondria.

Tom37 was identified during a screen for genes involved in mitochondrial phospholipid metabolism. An association between Tom37 and Tom70 has been shown. Additionally, while deletion of either gene does not affect cell viability, yeast cells lacking both are inviable [30]. Evidence for preprotein binding to Tom37 has not been demonstrated, nor is Tom37 required for facilitating the binding of preproteins to Tom70 [30, 31]. In contrast to other members of the Tom machinery, Tom37 was recently found to be peripherally associated with the mitochondrial outer membrane [31]. The role of Tom37 in mitochondrial preprotein import remains to be resolved.

A homologue of Tom70, termed Tom72, is also found in mitochondria but is expressed in low amounts under normal growth conditions. Tom72 seems to have little significant role in mediating mitochondrial protein import [32].

The structural organization of the receptor complexes described here should be regarded as a very dynamic system: it was shown that the association and dissociation of the receptors with the GIP can be influenced by Tom6 and Tom7 (see below). The receptors can also substitute for each other in their specificity for preproteins, indicating that they have both complementing and overlapping functions. Indeed, the deletion of either TOM20 or TOM70 genes leads to only partial import defects, while deletion of both genes is lethal [33]. Interestingly, however, this lethality can be overcome when the expression of Tom22 is increased in such cells [14, 34]. It seems that under normal cellular conditions, Tom22 requires the presence of at least one of these receptors for its biogenesis. Its increased expression in cells lacking both receptors overcomes such a requirement. Cells can survive with Tom22 as the sole receptor indicating that Tom22 is the central receptor required for the import of nearly all preproteins.

The GIP complex

Following binding to the Tom receptors, preproteins are subsequently transported across the outer membrane at the GIP. Dekker et al. [14] showed by blue native electrophoresis that the yeast GIP complex is a stable complex of about 400 kDa and contains the two major proteins Tom40 and Tom22 as well as the three small Toms—Tom5, Tom6, and Tom7.

Tom40 constitutes the channel of the import pore

Tom40 is an integral membrane protein almost completely embedded in the outer membrane. It spans the membrane several times by β-strands and has predicted structural similarity to porins which form metabolite-transmitting channels in the outer membranes of mitochondrial and bacterial outer membranes [35–38].

Recent studies have identified the components of the GIP complex that form the protein-translocating channel. By using recombinant purified Tom40, Hill et al. [39] were able to reconstitute this protein in liposomes. Electrophysiological measurements demonstrated that these proteoliposomes contain a cation-selective channel with an estimated pore size of about 22 Å. Such a pore size is sufficient to translocate a protein in an extended or α-helical confirmation. Even a loop could fit into such a channel but higher-structure elements cannot. Such experiments also revealed that this Tom40 channel is, by itself, in a mainly open state. Interestingly, it was further shown that the TOM channel of the outer membrane, containing additional components interacting with Tom40, has a high probability of being closed. This observation led to the conclusion that one (or more) of the other GIP components, or even yet unidentified factors, can serve as regulatory elements in gating the protein translocation channel.

Tom22 as organizer of the GIP complex

A recent study showed that Tom22 is not only crucial as an import receptor but also functions as an organizer of the translocase. This conclusion was based on studies comparing mitochondria from wild-type cells with those isolated from cells lacking Tom22 [40]. Such a *tom22Δ* cell line was growth defective, exhibited reduced mitochondrial import rates, and lacked mitochondrial DNA. When isolated mitochondria were subjected to native electrophoresis, it was found that the GIP complex had dissociated from the 400-kDa wild-type state into a 100-kDa core containing a Tom40 dimer along with Tom5, Tom6, and Tom7. This smaller complex represents the basic translocation unit and can arrest spanning preproteins. Electrophysiological measurements revealed that the TOM channel in *tom22Δ* mitochondria is mainly in an open state—similar to the channel activity of Tom40 alone (fig. 2). Since the wild-type TOM channel is in a mainly closed state [39], these findings suggest that Tom22 somehow regulates channel gating. The significance of such gating activity for the import of preproteins remains to be resolved.

How is the 400-kDa GIP complex stabilized by Tom22? It was shown that neither the removal of the cytosolic nor the IMS domain of Tom22 affects the stability of the GIP. Only the single trans-membrane domain of Tom22 is crucial for the stable association of this 400-kDa complex [40]. Thus Tom22 associates with the 100-kDa core complexes via its trans-membrane segment to form a larger complex, probably consisting of

three core complexes. Indeed, electron micrographs of the purified TOM complex from *Neurospora crassa* revealed two to three stain-filled pits which were interpreted as the protein translocation pores [13].

The small Tom proteins

The small Toms represent a group of integral membrane proteins of low molecular weight that are tightly associated with the GIP where they exert diverse functions which aid in optimization of preprotein translocation. Tom5 and Tom7 were identified by co-immunoprecipitation using antibodies directed against Tom40 [22, 41] while Tom6 was initially detected as a multicopy suppressor of mutant forms of the *TOM40* gene [42].

A small N-terminal part of Tom5 is exposed to the cytosol and contains a negative net charge. Antibodies against this domain inhibit protein import suggesting that Tom5 recognizes the positively charged presequence. In yeast strains where the TOM5 gene is deleted, recognition of preproteins by the receptors Tom20, Tom70, and Tom22 is unchanged; however, their transfer and insertion into the TOM channel is impaired. It was therefore concluded that Tom5 functions as a linker between the preprotein receptors and the GIP [22].

Tom6 and Tom7 are not directly involved in the translocation process but control the stability of the GIP complex in an antagonistic manner. Deletion of Tom6 leads to destabilization of the interaction between Tom40 and Tom22 indicating a role for Tom6 in GIP complex assembly [43]. This was directly observed via blue native electrophoresis, where a portion of the 400-kDa GIP complexes had dissociated into 100-kDa subcomplexes in *TOM6* deletion mutants [14]. This subcomplex contains Tom40, Tom5, and Tom7 but not Tom22, and is similar to the 100-kDa subcomplex found in Tom22-deficient strains. When Tom6 is added back, the 400-kDa complex can be restored [14].

In contrast, the absence of Tom7 seems to stabilize the interactions between members of the TOM machinery. Import of preproteins destined for the inner membrane or matrix is not significantly impaired by deletion of Tom7. However, optimal import of outer membrane proteins such as porins depends on the presence of Tom7. Hence Tom7 is proposed to function as a lateral opener of the translocation channel, facilitating the biogenesis of outer membrane proteins [14, 41].

What drives preproteins into mitochondria?

Translocation across the inner membrane strongly depends on the presence of the membrane potential ($\Delta\psi$) and the ATP-dependent action of mtHsp70 at the matrix side of the inner membrane. mtHsp70 is thought to function as a molecular motor which converts the energy stored in ATP into a pulling force for the import of preproteins [44, 45]. In contrast, no direct import driv-

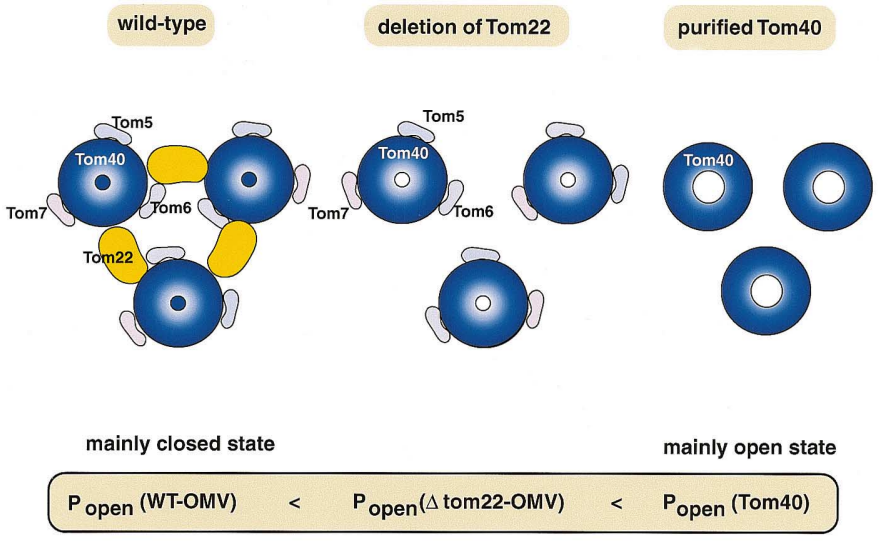

Figure 2. Schematic diagram depicting the architecture of the Tom40-containing complex in wild-type mitochondria (left), mitochondria lacking Tom22 (middle) or in reconstituted recombinant Tom40 (right), and the associated open probability of its channel as measured by electrophysiology [39, 40].

ing force for preprotein translocation across the outer membrane has been identified. However, a model termed the *acid chain hypothesis* has been postulated to explain such transport [18, 46]. This model is based on the fact that many of the import components of the translocation machinery contain acidic patches. Such negatively charged regions are found within the cytosolic domains of the receptors Tom70, Tom20, and Tom22 and could serve as cis-binding sites for the positively charged presequences of preproteins. Tom22 also possesses a negatively charged domain in the IMS thereby providing a trans-binding site for preproteins. A trans-binding site is also found in Tom40 although its nature is not yet known [47]. Additionally, Tim23 provides a small acidic domain located at the IMS side of the inner membrane which could serve as a docking point for the incoming protein chain. Komiya at al. [48] proposed that these various binding sites have different affinities for preproteins; the initial receptor has a relatively low affinity for preproteins while increasingly higher affinities are observed for the binding sites later in the pathway. The transfer of preproteins along this acid chain would enable the preprotein to be translocated across the outer mitochondrial membrane in a unidirectional manner.

A prerequisite for a mechanism according to the acid chain hypothesis is of course the presence of a positively charged presequence. How can preproteins that lack such presequences translocate across the outer membrane? An alternative pathway has been recently reported for the translocation of carrier preproteins destined for the inner mitochondrial membrane [4, 5]. These preproteins contain internal targeting signals and can accumulate at the trans side independently of the IMS domain of Tom22 and even in the absence of a $\Delta\psi$ [24]. As the carrier preproteins emerge from the GIP into the IMS, they are bound by members of a family of small Tim proteins (fig. 1). These small Tim proteins may even be involved in 'pulling' the carrier preproteins across the outer membrane before guiding them to the inner membrane [6–8].

Although there is now a clearer conception regarding protein translocation across the outer membrane, many efforts must still be made in order to understand the exact molecular principles of this process. In particular, establishment of reconstituted systems containing components of the Tom machinery will help to clarify unresolved mechanisms. Recent studies show that such analyses are now successfully underway [13, 39].

Most of the details presented in this review were obtained from studies using the fungal model system of *S. cerevisiae*. Based largely on sequence homologies to known components, Tom homologues in higher eukaryotes such as potato [49] and human have been reported [50–53]. A detailed database search using the *C. elegans* genome sequence has found members of the TOM family with sequence identities between 17% (Tom70) and 37% (Tom6) [54]. These findings indicate a conservation of the TOM machinery in preprotein import and will help us to understand the general principles involved in preprotein translocation.

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