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GRAPHICAL Review

Protein translocation in mitochondria: Sorting out the Toms, Tims, Pams, Sams, and Mia

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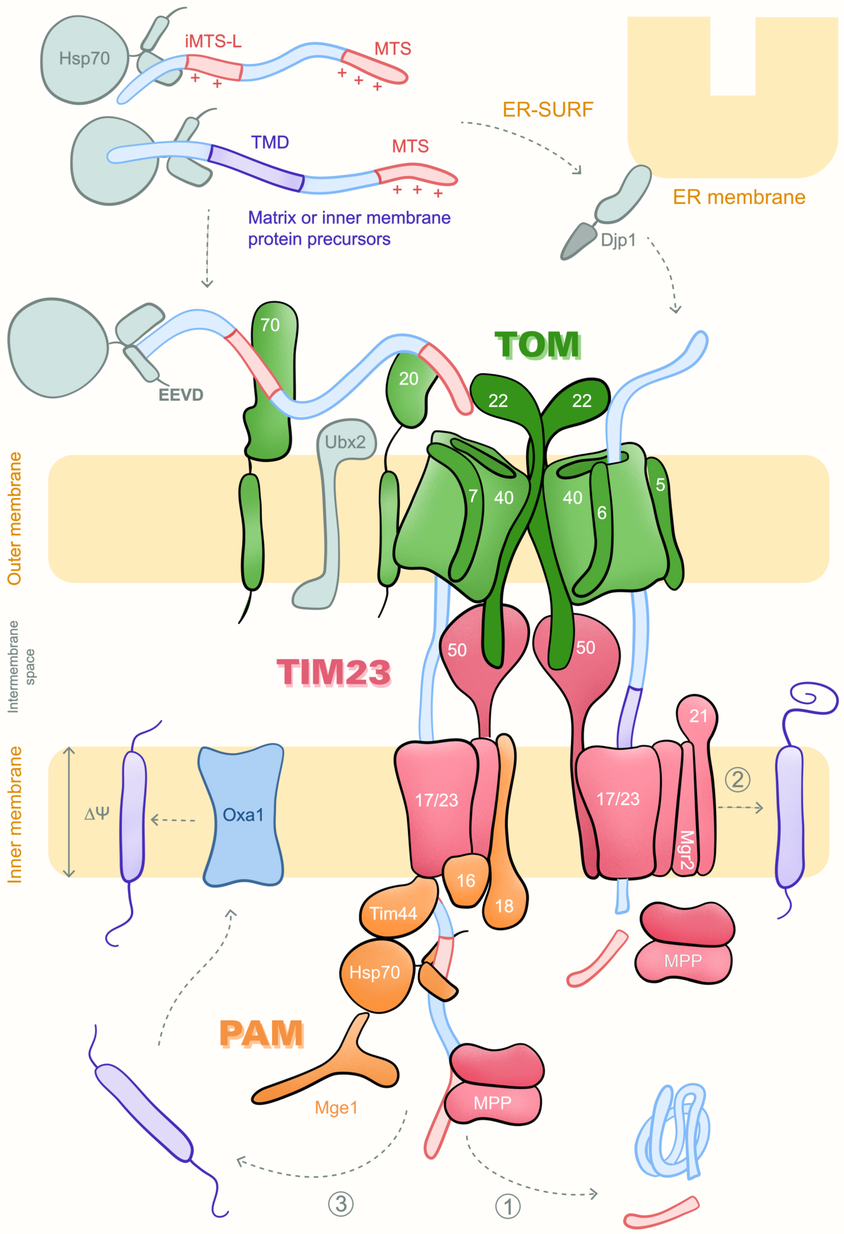
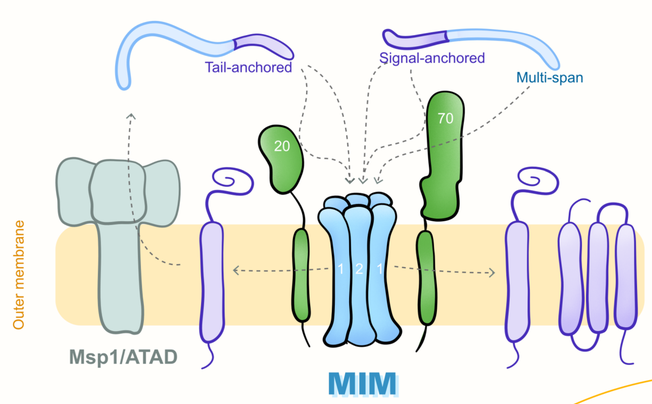
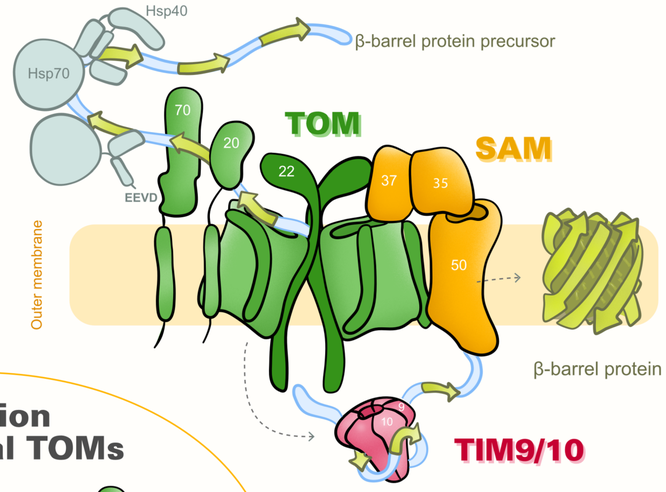
The matrix-targeting or TIM23 pathway

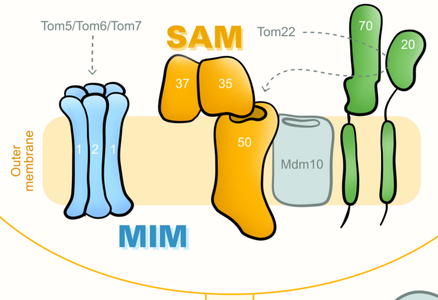


Insertion of outer membrane proteins

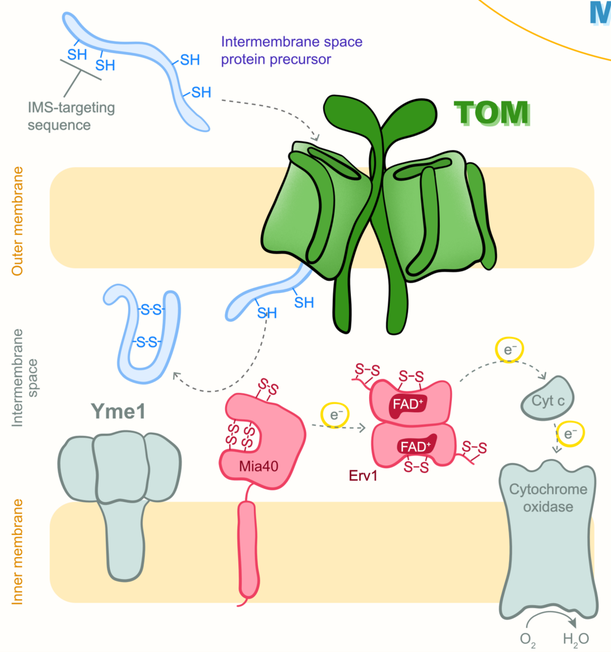




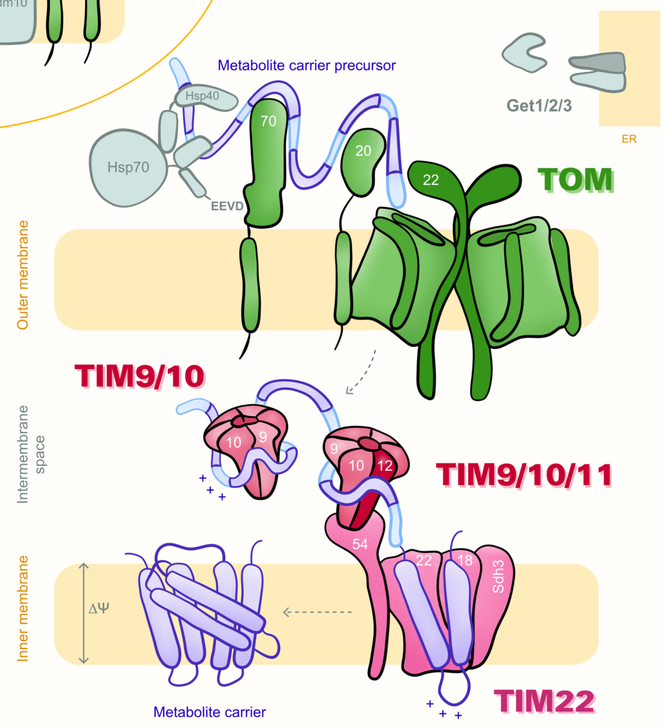
Insertion of α-helical TOMs



The MIA pathway (mitochondrial disulfide relay)



The carrier pathway (TIM22 pathway)



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GRAPHICAL Review

Mitochondrial protein import

Mitochondria contain 902 (yeast) to 1.136 (mouse, humans) verified proteins [1, 2]. Except for a very small number of mitochondrially encoded core components of the respiratory chain, mitochondrial proteins are encoded by nuclear genes and synthesized in the cytosol. Different **import pathways** direct proteins to their respective mitochondrial subcompartment (outer membrane, intermembrane space (IMS), inner membrane and matrix). Specific **targeting signals** in their sequence direct proteins to their target destination and allow the proteins to embark on their respective import pathway. The main import pathways are shown here on the poster and are introduced in the following, using the mitochondrial import system of the baker's yeast *Saccharomyces cerevisiae* as example. However, the mitochondrial import system of mammalian cells is highly similar and deviates only in minor aspects [3]. Even the mitochondrial import machineries of less closely related eukaryotes, such as plants [4] and trypanosomes [5], are very similar and adhere to the same general principles.

The main road: The presequence or TIM23 pathway

Approximately 60% of all mitochondrial proteins are synthesized with an N-terminal presequence. Presequences are 15-70 residues long and contain a matrix targeting signal (MTS) that has the potential to form an amphipathic helix with a positive and a hydrophobic surface. MTSs can be identified reliably by prediction programs [6]. MTSs are recognized on the mitochondrial surface by Tom20 and Tom70, two receptors of the **TOM complex**. Tom70 binds also internal MTS-like sequences in precursor proteins and recruits chaperones to the TOM complex [7-9]. Some proteins reach the TOM complex via the ER surface (ER-SURF) [10]. Proteins are passed through the beta-barrel protein Tom40 [11, 12] to the **TIM23 complex** where Tim50, Tim23 and Tim17 facilitate membrane-potential driven translocation across (①) or into (②) the inner membrane [13]. The **import motor or PAM complex** completes the translocation in an Hsp70-driven, ATP-dependent reaction [14]. The motor consists of the Hsp70 binding subunit Tim44 and regulatory proteins Pam16 and Pam18 which have DnaJ-like domains. In the matrix, the presequences are removed by the mitochondrial processing peptidase MPP. Some membrane proteins can be inserted from the matrix into the inner membrane by the Oxa1 insertase (③). Non-productive translocation intermediates are proteolysed in a process called **mitochondria-associated degradation (MAD)**. Ubx2 ubiquitinates import intermediates which are then extracted by Cdc48/p97/VCP and degraded by the proteasome [15].

Protein insertion into the outer membrane

About 10-15% of all mitochondrial proteins are located in the outer membrane. Outer membrane proteins lack presequences. Many outer membrane proteins have N- or C-terminal membrane anchors. The insertion of these proteins is catalyzed by Mim1 and Mim2 in yeast [16] or by MTCH2 in animals [17]. The outer membrane is characterized by the presence of beta-barrel proteins such as the very abundant protein porin (also VDAC, voltage-depenendent anion channel), Tom40, and Sam50. They are imported through the protein-conducting channel of **the TOM complex** into the IMS, where they bind soluble chaperones formed by small Tim proteins and then insert into the outer membrane by the Sam50 subunit of **the SAM complex** [18]. The AAA protein Msp1 (yeast) or ATAD1 (humans) serves as correction factor that extracts inappropriately inserted TA proteins that belong to the endoplasmic reticulum or to peroxisomes [19, 20].

The MIA pathway

About 15% of all mitochondrial proteins reside in the IMS. Many of these proteins are imported by the **mitochondrial disulfide relay** in a reaction that depends on the oxidation of cysteine residues. Internal cysteine-containing motifs called IMS targeting signal (ITS) or mitochondrial IMS sorting (MISS) signal serve as targeting signals [25, 26]. **Mia40** (CHCHD4 in humans) serves as essential import receptor for these proteins. Mia40 is maintained in the oxidized, functional state by the sulfhydryl oxidase Erv1 (augmenter of liver regeneration or ALR in humans) and cytochrome *c*. In humans, Mia40 forms a complex with the apoptosis inducing factor AIF [27]. The iAAA protease Yme1 degrades Mia40 substrates that lack or have incorrect disulfide bonds [28].

The carrier or TIM22 pathway

The inner membrane contains many carrier proteins (members of the mitochondrial carrier family, MCF or SLC25) that are characterized by six transmembrane domains and a common structure. They lack presequences and contain internal targeting signals referred to as carrier motifs [21]. Their translocation through the **TOM complex** is facilitated by Tom70 on the outer membrane and by hexameric complexes of small Tim proteins in the IMS [22]. The **TIM22 complex** inserts carriers into the inner membrane [21]. The membrane-embedded subunit Tim22 serves as insertase and is structurally related to Tim17 and Tim23 of the TIM23 complex [23]. Several accessory subunits are associated with Tim22 which are not conserved among eukaryotes. The import of carriers is dependent on the membrane potential but independent of mitochondrial ATP. The guided entry of tail-anchored proteins (GET) complex targets non-imported carrier proteins to the ER to protect cells against proteotoxic stress [24].

Final remark

The details of these import pathways were studied by importing a small number of model proteins into isolated yeast mitochondria. Such *in vitro* reconstitution experiments proved to be excellently suited to unravel the individual reactions of the translocation process across mitochondrial membranes. However, our understanding of the early reactions in the cytosol, such as the spatio-temporal organization of mitochondrial protein targeting under physiological intracellular conditions, is still very limited.

Abbreviations:

AAA, ATPases associated with different cellular activities; ALR, augmenter of liver regeneration; ATAD1, ATPase family AAA domain-containing 1; GET, guided entry of tail-anchored proteins; CHCHD4, coiled-coil-helix-coiled-coil-helix domain-containing protein 4; Hsp, heat shock protein; IMS, intermembrane space; MAD, mitochondria-associated degradation; ITS, IMS targeting signal; MCF, mitochondrial carrier family; MIA, mitochondrial intermembrane space import and assembly; MISS, mitochondrial IMS sorting signal; MTCH2, mitochondrial carrier 2; MPP, mitochondrial processing peptidase; MTS, matrix-targeting signal; PAM, presequence translocase-associated motor; SAM, sorting and assembly machinery; SLC25, solute carrier family 25; TIM, translocase of the inner membrane of mitochondria; TOM, translocase of the outer membrane of mitochondria; VDAC, voltage-dependent anion channel.

Components of the mitochondrial import pathways of yeast and humans.

	Yeast	Human	Function
The TOM complex	Tom70, Tom71	Tom70/TOMM70	Surface receptor
	Tom40	Tom40/TOMM40	Translocation pore, beta-barrel protein
	Tom22	Tom22/TOMM22	Multifunctional organizer
	Tom20	Tom20/TOMM20	Surface receptor
	Tom7	Tom7/TOMM7	
	Tom6	Tom6/TOMM6	
	Tom5	Tom5/TOMM5	
The SAM complex	Sam50 (Tob55)	Sam50/SAMM50	Translocation pore, beta-barrel protein
	Mdm10	- absent -	beta-barrel protein
	Sam37 (Mas37)	- absent -	
	Sam35 (Tob38)	- absent -	
	- absent -	Mtx1, Metaxin1	
	- absent -	Mtx2, Metaxin2	
The TIM23 complex (with PAM promoter)	Tim50	Tim50/TIMM50	IMS-exposed receptor
	Tim44	Tim44/TIMM44	Membrane anchor for Hsp70
	Tim23	Tim23/TIMM23	Inner membrane pore
	Tim21	Tim21/TIMM21	Regulatory subunit
	Tim17	Tim17a/TIMM17A, Tim17b/TIMM17B	Inner membrane pore
	Pam18 (Tim14)	DNAJC15 and DNAJC19	J protein
	Pam17	- absent -	
	Pam16 (Tim16)	Pam16/Magmas	J-like protein
	mtHsp70 (Ssc1)	HSPA9/Mortalin	Chaperone
	Mge1	mtGrpE/GRPEL1	Nucleotide exchange factor
The Tim22 complex and small Tims	- absent -	AGK	Acylglycerol kinase
	Tim54	- absent -	
	- absent -	Tim29	
	Tim22	Tim22/TIMM22	Translocation pore
	Tim18	- absent -	
	Sdh3	- absent -	
	Tim8, Tim13	TIMM8A/DDP1, TIMM8B, TIMM13	IMS chaperone complex
	Tim9, Tim10, Tim12	TIMM9, TIMM10A, TIMM10B	IMS chaperone complex
Further	Mim1	- absent -	Outer membrane insertase
	Mim2	- absent -	Outer membrane insertase
	- absent -	MTCH2	Outer membrane insertase
	Oxa1	Oxa1/OXA1L	Inner membrane insertase
	Mia40	Mia40/CHCHD4	Oxidoreductase
	- absent -	AIFM1	Membrane anchor of Mia40
	Erv1	ALR/GFER	Sulfhydryl oxidase
	Mas1	PMPCB	Mitochondrial processing peptidase, beta subunit
	Mas2	PMPCA	Mitochondrial processing peptidase, alpha subunit
	Mip1 (Oct1)	MIPEP	Mitochondrial intermediate peptidase

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