Common ground for protein translocation: access control for mitochondria and chloroplasts

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Abstract | Mitochondria and chloroplasts import the vast majority of their proteins across two membranes, and use translocases of the outer membrane as an entry gate. These translocases interact with the incoming precursor protein and guiding chaperone factors. Within the translocon, precursor-protein receptors dock to a central component that mediates both transfer through a cation-selective channel and initial sorting towards internal subcompartments. Despite these similarities, the mode of translocation differs between the two organelles: in chloroplasts, GTP-binding and hydrolysis by the receptors is required for transport, whereas in mitochondria passage of the preprotein is driven by its increasing affinity for the translocase subunits.

Endosymbiosis
Endosymbiosis is the process in which a free-living bacteria — the ancestral endosymbiont — was enclosed by a cell and, during evolution, became integrated into the cellular network. By transfer of most of its genetic content to the host, the nucleus lost its independence and became an organelle.

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Mitochondria and plastids both originate from the incorporation of a prokaryote by eukaryotic ancestor cells, an event called endosymbiosis. It is believed that the engulfment of a purple bacterium led to the development of mitochondria[1], whereas plastids emerged from the incorporation of a cyanobacterial progenitor by a eukaryotic cell that already contained mitochondria[2]. Both organelles are crucial for cell survival. Mitochondria, which exist in all eukaryotic cell types, are important for apoptosis, lipid and fatty-acid metabolism, haem biosynthesis, respiratory ATP production and iron–sulphur cluster assembly[3,4]. By contrast, plastids are a plant-specific family of organelles that differentiate into organelles of distinct morphology and function depending on the plant organ and stage of development[5]. The most prominent and best-investigated plastid type is the chloroplast, which contains the thylakoid membrane, where oxygenic photosynthesis takes place[6]. Thus, among many other functions, plastids are central to carbon fixation.

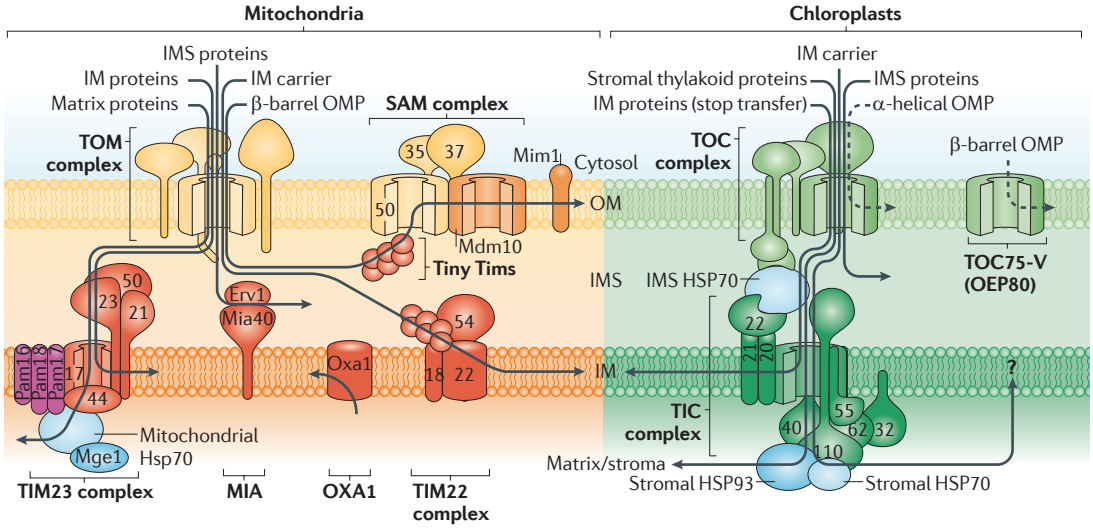
Fulfilling these diverse roles requires about 1,000 different proteins in the mitochondria of the budding yeast *Saccharomyces cerevisiae* and 2,000 proteins in plant chloroplasts[7,8]. However, both mitochondria and plastids contain genomic information encoding only a comparatively small number of proteins; during the evolutionary development of both organelles most of the endosymbiont's genetic information was transferred to the host genome. Therefore, more than 95% of mitochondrial and plastid proteins are encoded by the nuclear genome, synthesized on cytosolic ribosomes and imported into the target organelle.

In this Review, we compare the fundamental principles of targeting to and translocation into mitochondria and chloroplasts by translocases of the outer membranes of mitochondria (TOM complex) and chloroplasts (TOC complex), and their functional and architectural similarities and differences. Unless stated otherwise, we refer throughout to studies of the TOM complex in yeast and the TOC complex in peas (*Pisum savitum*) or *Arabidopsis thaliana*. We discuss how they are equipped to handle a broad range of substrates and how specific features, such as the receptor set-up and the active sites of the translocons, ensure proper sorting towards different subcompartments. Both translocases contain a multifunctional component that coordinates precursor-protein transfer towards the translocation pore and controls sorting and communication with subsequent translocases. In this way, the translocases of the outer membrane mediate subsequent steps of translocation. We propose that this central organizer of the outer-membrane translocases is a common characteristic of protein translocation into endosymbiotically derived organelles.

The general principle of protein translocation

Protein uptake into a cell organelle is common and essential for all eukaryotic cells. This can occur by two mechanisms: co-translational import, which requires protein translocation to be tightly coupled to translation[9]; or post-translational import, in which protein synthesis

Box 1 | **Intra-organelle transport in mitochondria and chloroplasts**



In both mitochondria and chloroplasts, precursor proteins are transferred across the outer membrane (OM) by the translocase of the outer membrane of mitochondria (TOM) complex (yellow) or the translocase of the outer envelope membrane of chloroplasts (TOC) complex (green) and are subsequently sorted to different locations within the organelle[14,15,17].

In mitochondria, the translocase of the inner membrane of mitochondria 23 (TIM23) complex (orange) acts in cooperation with the presequence-associated motor (PAM) complex (purple) to mediate protein transfer into the matrix[14]. The mitochondrial heat-shock protein 70 (Hsp70) provides the driving force within the PAM module and is regulated by its co-chaperones Pam18/16 and Mge1. In its PAM-free form, the TIM23 complex mediates the sorting of precursor proteins with a 'stop' signal into the inner membrane (IM)[14]. Oxa1 (which is related to the bacterial protein YidC and the chloroplast Alb protein)[136] facilitates protein insertion from the matrix into the inner membrane, whereas the TIM22 complex assembles carrier proteins with internal signal peptides into the inner membrane[14,15]. The tiny Tim proteins guide β-barrel precursor proteins across the intermembrane space (IMS) to the sorting and assembly machinery (SAM) that catalyses integration into the outer membrane (OMP; outer membrane protein). The SAM components can also associate with Mdm10 and Mim1 to facilitate the biogenesis of outer membrane proteins. The mitochondrial IMS import and assembly machinery (MIA), which consists of Mia40 and Erv1, stimulates the import and assembly of IMS proteins with a characteristic cysteine motif[137]. Alternatively, proteins are first sorted into the inner membrane by the TIM23 translocase and subsequently released into the intermembrane space by proteolytic cleavage of the membrane anchor.

In the case of chloroplasts, much less is known about sorting control[17,48,138]. Chloroplast precursor proteins are imported into the stroma by the translocase of the inner envelope membrane of chloroplasts (TIC) complex. The import motor comprises the stromal HSP93 (also termed ClpC) and stromal HSP70 that dock onto TIC40/TIC110 in the TIC complex[17,139–141]. There are two routes into the inner membrane: the 'stop-transfer' route through the TIC translocon, and a conservative sorting pathway that involves transport into the stroma followed by membrane integration from the stromal side. Toc75-V (also known as OEP80) may also be important for translocation of β-barrel proteins into chloroplasts[101]. So far, the import pathways of α-helical outer membrane proteins of mitochondria and chloroplasts, and of intermembrane space proteins of chloroplasts are poorly understood.

is not mechanically linked to protein translocation. In the latter case, cytosolic chaperones guide the precursor to the target organelle[9]. Despite the structural and functional diversity of protein-translocation machineries in different organelles, there are some common principles: cytosolic synthesis of a precursor protein containing an organelle-specific signal; transport to the target membrane in an import-competent form by cytosolic factors such as the signal-recognition particle (SRP) for the co-translational pathway[9,10] and chaperones for the post-translational pathway; recognition by receptors on the organelle surface; transport across the membrane by a translocation channel; and an energy force that drives translocation[9,11]. Finally, after import, processing and folding of the precursor protein takes place inside the target organelle. However, some translocases — in peroxisomes, the nucleus or the twin-arginine-motif-dependent TAT apparatus of bacterial plasma and thylakoid membranes — import precursor proteins that are already folded[11,12]. These import machineries have a pore of variable size that accommodates the dimensions of the folded precursor protein upon its recognition[11]. Such import systems have not been described for the envelope membranes of mitochondrial and chloroplast membranes.

Generally, mitochondrial and chloroplast proteins are imported post-translationally. In mitochondria and chloroplasts, several protein machineries mediate transport of precursor proteins into different subcompartments (BOX 1; FIG. 1). The translocases of the TOM and TOC complexes provide the entry gate for most precursor proteins and mediate the initial sorting steps towards the organelles' subcompartments. Thus, the translocases of both organelle types recognize and

Thylakoid membrane
A component of chloroplasts, the thylakoid membrane is a specialized membranous compartment where photosynthesis occurs.

Oxygenic photosynthesis
Oxygenic photosynthesis is the conversion of carbon dioxide and water into organic compounds, especially sugars, and oxygen by the thylakoid and stromal enzymes, including the photosystems.

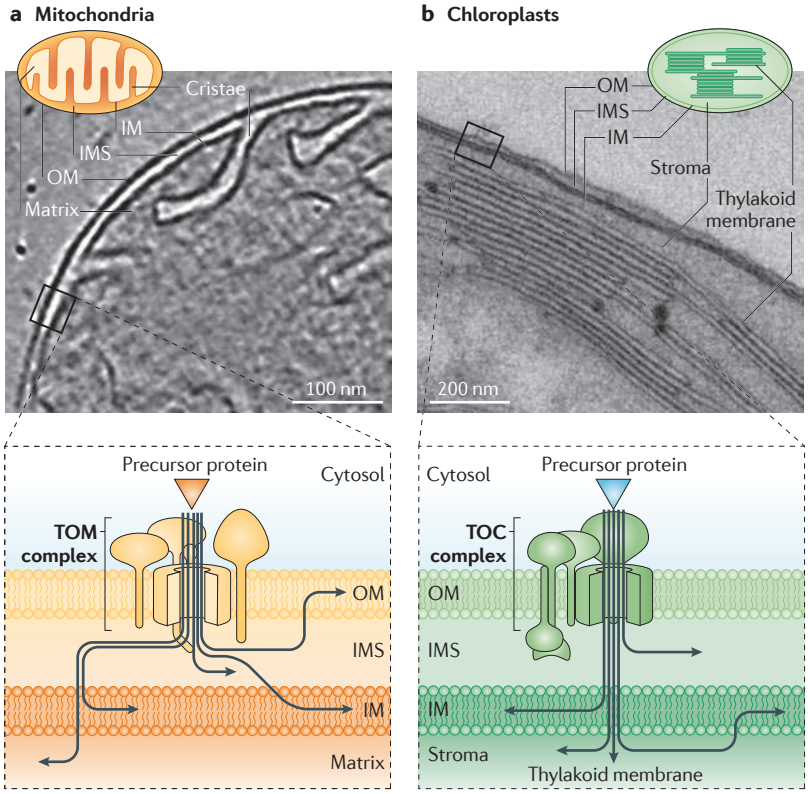


Figure 1 | **Protein transport into mitochondria and chloroplasts.** The ultrastructure of mitochondria and chloroplasts is shown schematically and by electron microscopy. Both organelles are bounded by two membranes, the outer membrane (OM) and inner membrane (IM). **a** | The mitochondrial inner membrane can be separated into the 'inner boundary membrane' region that lies close to the outer membrane and the large invaginations, or cristae, where protein complexes form the respiratory chain. Mitochondria also have two aqueous compartments, the matrix and the intermembrane space (IMS), the latter of which is continuously connected with the cristae lumens. **b** | Chloroplasts contain an additional compartment, the thylakoid membrane system, which harbours the protein complexes that drive photosynthesis. Chloroplasts therefore have three aqueous compartments: the thylakoid lumen, the stroma and the IMS. For most precursor proteins, translocation into mitochondria (**a**) and chloroplasts (**b**) is mediated by the translocase of the outer membrane of mitochondria (TOM) complex and the translocase of the outer membrane of chloroplasts (TOC) complexes, respectively. Subsequently, these translocases cooperate with other sorting machineries (not shown) to mediate protein targeting to distinct locations within the organelle, including the IMS, the inner membrane, the matrix of mitochondria or the stroma or thylakoid membranes of chloroplasts. For a detailed description of these pathways, see BOX 1. The electron microscopy images shown are of a mitochondrion from *Solanum tuberosum* (**a**) and a chloroplast from *Arabidopsis thaliana* (**b**), and were provided by B. Daum, F. Joos and W. Kühlbrandt of the Max Planck Institute for Biophysics, Frankfurt, Germany.

transport precursor proteins with diverse topologies, ranging from soluble proteins of the intermembrane space, the mitochondrial matrix or of the chloroplast stroma to membrane-bound proteins with single or multiple transmembrane domains.

Targeting to the organelle

Precursor proteins destined for import into mitochondria or chloroplasts are translated on cytosolic ribosomes and are targeted, with the help of chaperones, to the cell organelle. The targeting requires specific signals within the synthesized precursor, and receptor proteins on the organelle surface that recognize such signals, to confer high fidelity on the transport process. Most signals have important and unique features that target proteins specifically to mitochondria or to chloroplasts. However, in the plant cell about 50 proteins are synthesized with an ambiguous targeting signal that allows localization to both organelles[13].

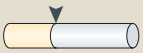
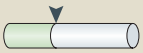
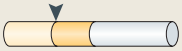
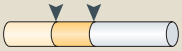
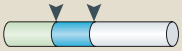
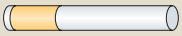
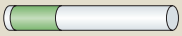
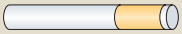
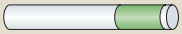
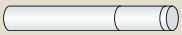
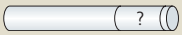
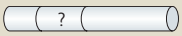
Signals for defining target specificity. Mitochondrial or chloroplast precursor proteins are synthesized on cytosolic ribosomes and contain either a cleavable or an internal, non-cleavable, signal sequence (TABLE 1). Most precursor proteins destined for import into the mitochondrial matrix or the stroma of chloroplasts are translated with an amino-terminal extension called a presequence or transit peptide, respectively[14–17]. This sequence comprises the signal information sufficient for proper targeting towards, and recognition on, the cell organelle surface and is cleaved off after successful protein translocation across the two membranes by processing peptidases[18,19]. Both types of presequence have an overall positive charge and a propensity to form an amphiphilic α-helix. The variable length and divergent primary structure of chloroplast and mitochondrial signals suggest that it is not a specific sequence motif but rather a certain structural or physicochemical feature that is recognized by import receptors. Mitochondrial- and chloroplast-targeting signals differ in their hydrophobicity and the starting point of the amphiphilic α-helix[20]. In addition, mitochondrial signals are enriched in arginines that form part of the recognition sites for the processing peptidase[20,21]. By contrast, the chloroplast-targeting signal is enriched in hydroxylated amino acids, which in some cases can be phosphorylated by a cytosolic kinase[22]. In plant cells, these differences in the targeting information are sufficient to drive proper targeting to either mitochondria or chloroplasts.

Chloroplast proteins of thylakoid membranes are synthesized with a 'bipartite' signal, which provides information for both chloroplast targeting and subsequent sorting in the stroma to the thylakoid membrane[16]. Similarly, a hydrophobic stretch following the signal sequence can mediate sorting into the inner membrane of mitochondria and chloroplasts[14,17]. In both organelles, a few precursor proteins are processed by a peptidase complex at the inner membrane and then released into the intermembrane space.

In addition, several non-cleavable signals have been described for chloroplasts and mitochondria (TABLE 1). In both, proteins of the outer membrane with α-helical membrane anchors carry their targeting information in the transmembrane domain and charged residues in the flanking regions[23,24]. Mitochondrial β-barrel proteins require a conserved motif within the last β-strand (termed a β-signal) for integration into the outer membrane[25]. Such a signal has not yet been found in the β-barrel proteins of chloroplasts but it is very likely that one exists. The targeting information of mitochondrial inner-membrane carrier proteins is localized in hydrophobic stretches, whereas a canonical cysteine-

Amphiphilic α-helix
An amphiphilic α-helix is a helix in which one side is composed of hydrophobic amino acids and the other of hydrophilic amino acids.

Table 1 | **The import signals of mitochondrial and chloroplast proteins**

Mitochondria	Chloroplasts	Signal	Destination
Cleavable signal sequences			
		Classic presequence	Mitochondrial matrix or chloroplast stroma
		Presequence and non-cleavable hydrophobic sorting signal	Mitochondrial or chloroplast inner membrane
		Presequence and cleavable hydrophobic sorting signal	Mitochondrial intermembrane space or chloroplast thylakoid lumen
Non-cleavable signal sequences			
		Outer-membrane protein (OMP) with signal anchor	Mitochondrial or chloroplast outer membrane
		OMP with internal anchor	Mitochondrial or chloroplast outer membrane
		OMP with carboxy-terminal anchor	Mitochondrial or chloroplast outer membrane
		OMP with multiple anchors	Mitochondrial or chloroplast outer membrane
		β-barrel OMP with β-signal	Mitochondrial or chloroplast outer membrane
		Inner membrane carrier with multiple signals	Mitochondrial inner membrane
		Inner membrane protein with internal presequence-like signal	Mitochondrial inner membrane
		Intermembrane-space protein with Cys-rich motif	Mitochondrial inner membrane
		Signal for intermembrane-space targeting	Chloroplast intermembrane space

rich motif is crucial for correct transport of intermembrane-space proteins[14,15,24]. In chloroplasts, many inner-membrane proteins contain a classic transit peptide and only a few proteins have been described in the intermembrane space and the inner membrane that have non-cleavable targeting signals[17]. However, proteomic analyses indicate that several chloroplast proteins do not contain typical transit peptides[26]; their localization needs to be confirmed given reports that some of these proteins are attached only to the outer envelope membrane[27].

Transport to endosymbiotically derived organelles. Generally, it is thought that protein import into mitochondria and chloroplasts occurs post-translationally and is mediated by soluble factors (FIG. 2). These soluble factors include chaperones such as heat-shock protein 70 (Hsp70), which binds to many precursor proteins to keep them in an unfolded import-competent state[15]. Hsp70 can act alone or in cooperation with other cytosolic factors such as 14-3-3 proteins or Hsp90 (REFS 28–33). 14-3-3 proteins bind to phosphoserine-containing consensus motifs to modulate biochemical processes such as signal transduction or, as described here, protein translocation[34]. For chloroplast precursor proteins, a 'guidance' complex containing Hsp70 and a 14-3-3 protein forms after phosphorylation of the transit peptides[29]. Phosphorylation of mitochondrial precursor proteins has not been shown, but mitochondrial import-stimulating factor (MSF), which belongs to the 14-3-3 protein family, stimulates protein translocation into mitochondria[28]. In yeast, Hsp70 delivers precursor proteins such as inner-membrane carrier proteins that are prone to aggregation at the mitochondrial surface[31]. In mammals and plants, Hsp70 and Hsp90 cooperate to target such hydrophobic precursors to mitochondria or to chloroplasts[31,33]. Both Hsp70 and Hsp90 are recognized by a specific tetratricopeptide repeat (TPR) motif of a peripheral receptor subunit on the according target membrane, and 14-3-3 proteins also interact with translocase receptors[28,31,33].

In addition to these chaperones, several other factors can target a subset of precursor proteins to mitochondria and/or chloroplasts[30,35–37] (FIG. 2). For example, the ankyrin-rich protein AKR2A mediates the biogenesis of chloroplast outer membrane proteins with an α-helical membrane anchor[36,37]. Similarly, the arylhydrocarbon-receptor-interacting protein (AIP) stimulates the import of preornithin transcarbamylase into human mitochondria *in vivo*[30]. So far, it is not clear whether these factors are specifically required for the biogenesis of a small subset of substrates or whether they have a more general role in protein targeting to mitochondria and/or chloroplasts (FIG. 2), or whether they are indeed specific for targeting to one organelle[38].

β-barrel proteins
β-barrel proteins are membrane proteins that are typically found in the outer membrane of mitochondria, of chloroplasts and of Gram-negative bacteria. These proteins form a membrane-inserted barrel composed of β-strands.

14-3-3 proteins
Proteins that are expressed in eukaryotic cells and that bind preferentially to phosphorylated regions in diverse proteins involved in signal transduction and protein translocation.

Tetratricopeptide repeat
(TPR). A structural motif, found in a wide variety of proteins, that is composed of 34 amino acids. TPRs are involved in intra- and inter-molecular interactions.

Ankyrin
Ankyrin repeats are structurally but not functionally conserved units of 33 amino acids that consist of two α-helices separated by a loop, and comprise one of the most common structural motifs identified in bacterial, archaeal and eukaryotic proteins.

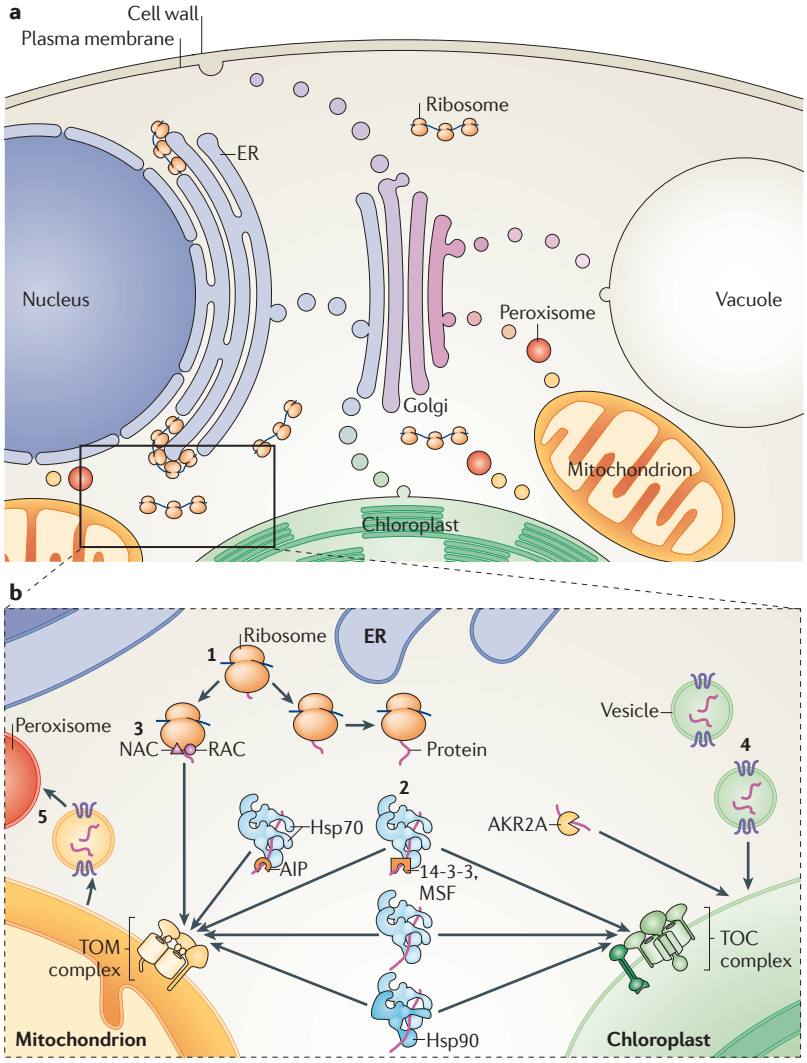


Figure 2 | **Transport of precursor proteins from the cell cytosol into mitochondria and chloroplasts. a** | The organelle structure of a typical plant cell is shown, including the nucleus, vacuole, peroxisomes, mitochondria, the endoplasmic reticulum (ER) and Golgi. Protein synthesis occurs on cytosolic ribosomes, and proteins are then sorted to various organelles, including mitochondria and chloroplasts. The box depicts the region shown in **b**. **b** | Most protein transport into mitochondria and chloroplasts occurs post-translationally. Precursor proteins are synthesized on cytosolic ribosomes (**1**) and are subsequently targeted to an organelle by cytosolic factors (**2**). Heat shock protein 70 (Hsp70) has a central role in targeting precursor proteins to mitochondria and chloroplasts. It acts alone or in association with cofactors such as Hsp90, 14-3-3 and arylhydrocarbon-receptor-interacting protein (AIP). The mitochondrial stimulating factor (MSF) promotes targeting of precursor proteins in the absence of Hsp70. The ankyrin-rich protein AKR2A guides chloroplast outer-membrane proteins to their target membrane. In some cases, there is a close proximity between translation and transportation into mitochondria (**3**). Factors such as the nascent-associated polypeptide complex (NAC) and ribosome-associated complex (RAC) have been reported to stimulate precursor protein import. For chloroplasts, it has been proposed that a subset of precursor proteins can be targeted from the ER by vesicle trafficking (**4**). Similarly, vesicular trafficking has been shown from mitochondria to peroxisomes (**5**).

The synthesis and transport of several mitochondrial precursor proteins might be coordinated at the organelle surface (FIG. 2). mRNA molecules encoding mitochondrial preproteins and a fraction of cytosolic ribosomes co-localize with mitochondria[39,40], and this association depends on the interaction of a receptor of the TOM complex with the translated mitochondrial presequence[41]. This view is supported by the finding that ribosome-associated factors such as nascent-associated polypeptide complex (NAC) and ribosome-associated complex (RAC) can stimulate precursor-protein import[35]. Future work is needed to clarify whether, in some cases, precursor-protein synthesis and import into mitochondria is coupled. So far, such a system has not been described for chloroplasts.

Finally, proteins can be exchanged between organelles (FIG. 2). Proteins are transported from the endoplasmic reticulum (ER) to chloroplasts or from mitochondria to peroxisomes by vesicle trafficking[42,43]. Moreover, a molecular tether has been described between mitochondria and the endoplasmic reticulum that consists of the ERMES complex (ER–mitochondria encounter structure complex) in yeast mitochondria and mitofusin 2 in human mitochondria[44,45]. Similarly, contact sites have been reported between chloroplasts and the endoplasmic reticulum[46]. Whether proteins are transported by these connections is not yet known.

Protein translocation across the outer membrane

The TOM and TOC complexes catalyse the transfer of precursor proteins across the outer membrane of mitochondria and chloroplasts, respectively. Both complexes consist of receptor proteins that act on the cytosolic face (Tom20, Tom70 and Tom22; and Toc34, Toc64 and Toc159) and the pore-forming proteins Tom40 and Toc75 (REFS 14,15,47,48) (FIG. 3a). In addition, the small Tom proteins Tom5 and Tom6 are involved in the assembly of the TOM machinery, and Tom6 and Tom7 are required for its stability[14,49,50]. Moreover, Tom5 has a role in the transfer of precursor proteins from Tom22 to the translocation pore[14,51,52]. Similar components have not yet been identified in the TOC complex. In plants, there are multiple isoforms of TOM and TOC components[53,54] and, at least for the TOC-receptor isoforms, it is proposed that they might exhibit different substrate specificity — for example, for photosynthetic versus housekeeping substrates — and that they might be coordinated into different TOC complexes[55–57]. Furthermore, although some plant TOM-receptor subunits show structural features that are distinct from those of their yeast homologues[58], the overall architecture and mechanism of plant and human TOM complexes seems to follow similar principles to those of their yeast counterparts.

Both translocases have to recognize and transport precursor proteins with diverse topologies, including proteins with a β-barrel structure, single and multiple transmembrane α-helixes, and soluble proteins of the intermembrane space and of the mitochondrial matrix, the chloroplast stroma or the thylakoid lumen. After transfer across the outer membrane, precursor proteins are sorted to different subcompartments of the cell organelle by several specific protein machineries (BOX 1). As a result, the TOM and TOC complex have to communicate with translocation factors and precursor proteins on both sides of the membrane.

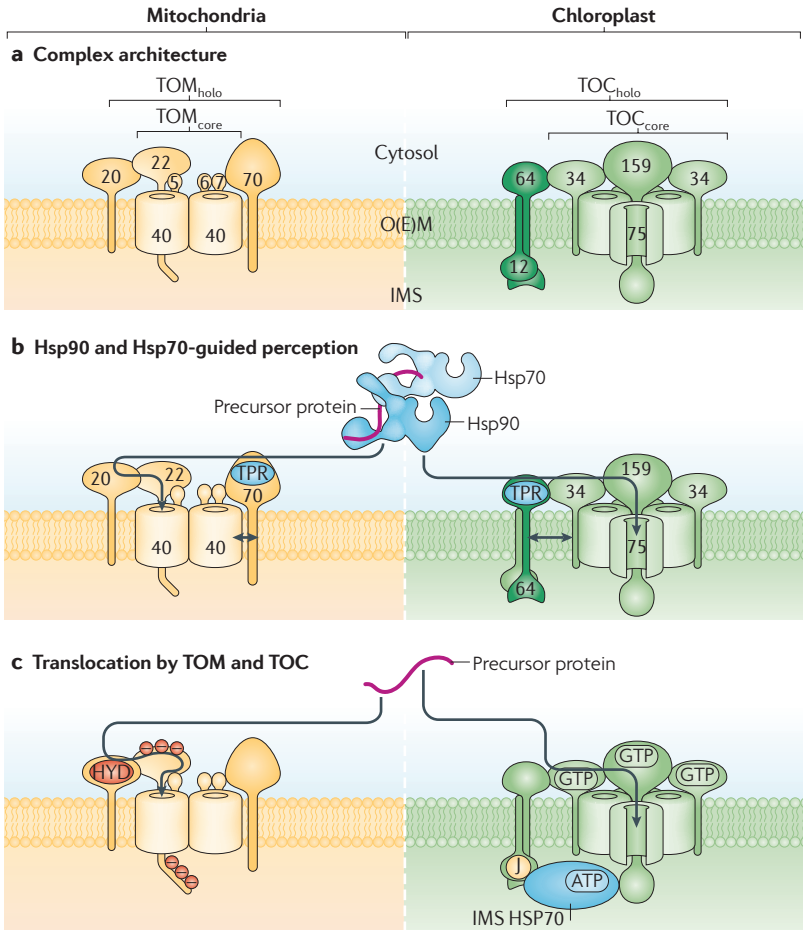


Figure 3 | **The translocon of the outer membrane of mitochondria and chloroplasts.** **a** | The translocase of the outer membrane of mitochondria (TOM) complex consists of the pore-forming Tom40 protein, the receptor proteins Tom70, Tom20 and Tom22 as well as the small Tom proteins (Tom5, Tom6 and Tom7) that are involved in regulation of complex stability. Tom70 and Tom20 are more loosely attached to the TOM_{core} complex, and together these form the TOM_{holo} complex. The translocase of the outer membrane of chloroplasts (TOC complex) is built by the pore-forming Toc75 protein, the receptor proteins Toc64, Toc34 and Toc159. Toc12 and Toc64 are not components of the TOC_{core} complex, but associate with this through Toc34. IMS, intermembrane space; O(E)M, outer (envelope) membrane. **b** | Many precursor proteins are delivered to the translocon by chaperones of the heat-shock protein 70 (Hsp70) and/or Hsp90 type. Hsp70 and/or Hsp90 guide hydrophobic precursor proteins to the Tom70 receptor in mitochondria or the Toc64 receptor in chloroplasts. The chaperones dock directly to the tetratricopeptide repeat (TPR) domain of these receptors, which are peripherally associated with the translocon. In the case of mitochondria, the precursor is likely to be subsequently delivered by Tom22 to the translocation pore. Some Tom70-dependent hydrophobic precursors also contain a cleavable presequence that is recognized by the receptor protein Tom20. In chloroplasts, the precursor protein first passes from Toc64 to Toc34, before transfer to Toc159 and then translocation by Toc75. Tom70 and Toc64 are loosely attached to the core translocon. This association might be stabilized during precursor transfer (shown by double-headed arrows). **c** | The TOM and TOC complexes have distinct modes of translocation. According to the 'increasing affinity' model (also termed the 'acid bristle' theory), an increasing density of negative charges on Tom22 drives the import through the TOM complex. Tom20 contains a hydrophobic pocket (HYD, hydrophobic-binding motif) that recognizes the signal in the precursor protein. Positively charged residues in the precursor protein then interact with negatively charged acid residues in the cytosolic domain and the intermembrane space region of Tom22 to drive translocation. The TOC complex contains two GTP-regulated receptors, Toc34 and Toc159, and GTP binding and hydrolysis by these receptors can stimulate precursor-protein import. Hsp70 localized in the IMS might provide additional driving force by ATP consumption for the translocation process and could be regulated by the DnaJ or 'J' domain of Toc12.

Architecture and dynamics of outer-membrane translocases. For each organelle, a central complex (TOM_{core} and TOC_{core}) has been assigned, on the basis of its resistance to detergent (FIG. 3a). The TOM_{core} complex consists of Tom40, Tom22 and the small Tom proteins Tom5, Tom6 and Tom7; the TOC_{core} complex consists of Toc159, Toc34 and Toc75. The receptor proteins Tom70 and Tom20, and Toc64, are more loosely associated[59,60].

From electron microscopy analysis, the TOM complex seems to contain two or three pore-forming regions, whereas the TOC complex has four[61–63]. Interestingly, the isolated TOM_{core} complex, which lacks Tom20, forms only two pores, indicating that Tom20 is important for normal architecture of the TOM complex[59,62]. In yeast mitochondria, the stability of the complex depends on Tom22 and the small Tom proteins[49,50,64]. Similarly, the receptor protein Toc34 affects the stability of the TOC complex, but through distinct mechanisms. First, its GTPase activity has been proposed to regulate dimerization of Toc34 and Toc159 during the translocation process[65–67], suggesting that the dynamics of the TOC complex are GTP-sensitive. Second, phosphorylation of Toc34 induces the disassembly of Toc34 from the TOC_{core} complex *in vitro*[68]. The significance of Toc34 *in vivo* phosphorylation is not yet clear, but may be important at very early developmental stages or under environmental conditions not yet identified[69,70]. Thus, the overall architecture of the outer-membrane translocase in mitochondria and chloroplasts differs in terms of the number of pores, but both rely on specific interactions between receptors and the core translocase complexes. On the basis of the data so far, the TOM complex seems rather stable, whereas the interaction of TOC subunits — at least that of Toc34 — is affected by phosphorylation and GTPase activity.

Two peripheral receptors in the receptor platform. The receptor-protein arrangement of the TOM and TOC complexes is similar (FIG. 3a) and, for both mitochondria and chloroplasts, precursor proteins bind here by two main routes. In many cases, Hsp70/Hsp90-containing complexes guide precursor proteins to the peripherally associated Tom70 or Toc64 subunits[31,33] (FIG. 3b). However, most precursor proteins are targeted directly to the presequence receptor Tom20 or Toc34 of the translocons[65,71] (FIG. 3c). Thus, the receptor subunit that is engaged by the precursor is determined by the import signal and by whether the precursor is bound to a cytosolic chaperone.

Tom70 and Toc64 contain a clamp-type tetratricopeptide repeat (TPR)[72] domain that is exposed to the cytosol and is required for docking of the Hsp70/Hsp90 chaperones[31,33,73] (FIG. 3b). In addition, Toc64 has an amidase-like domain that is involved in precursor-protein binding on the intermembrane-space site[74]. The functional similarity of Toc64 and Tom70 is further supported by the endogenous composition of the mitochondrial translocase in plants, in which a Toc64 isoform replaces Tom70 (REF. 75). The spectrum of proteins that are targeted to Tom70 or Toc64 includes those with internal signals, as well as those with cleavable presequences[33,76].

After association with Tom70 or Toc64, proteins are targeted towards the TOM_{core} or TOC_{core} complexes, respectively, for translocation. In the case of precursor proteins with cleavable signals, Tom70 binds to the mature part of the precursor protein, whereas Tom20 recognizes the presequence[76]. Similarly, chloroplast precursor proteins are transferred from Toc64 to the Toc34 receptor[33]. Thus, Tom20 and Tom70, as well as Toc64 and Toc34, have overlapping substrate specificity. However, because neither Tom70 nor Toc64 is essential for cell survival[77,78], Hsp70/Hsp90-guided precursor proteins must also be able to directly engage the Tom20 and Tom22 or Toc34 receptors. By contrast, deletion of Tom20 causes a severe growth defect in yeast and the knockout of both isoforms of TOC34 in *A. thaliana* is lethal[79–81], suggesting that TOC64 and Tom70 cannot fully compensate for the function of these receptors.

The scaffold role of Toc159 and Tom22. In both the TOM and TOC complexes, Tom20 and Tom22, as well as Toc34 and Toc159, bind to the precursor protein in a coupled manner[65,82,83]. For example, while Tom22 recognizes charged regions in the presequence of a precursor protein, Tom20 preferentially binds to hydrophobic stretches of the precursor[71,84,85]. Both Toc159 and Tom22 mediate the transfer of substrates to the translocation pore. They also coordinate the association with other receptor subunits and so are crucial to establishing a receptor platform: Tom22 provides the docking site for Tom20 and Tom70 (REF. 64), whereas Toc159 is in close contact to Toc34 (REFS 65,66). In turn, Tom20 and Toc34 are required for the assembly of the respective counterpart Tom22 or Toc159 into the translocon, respectively[86,87]. Consistent with this central role of Tom22 and TOC159, their deletion leads to a strong growth defect in yeast and *A. thaliana*, respectively, and largely blocks protein import into each organelle[64,88].

Control of the translocation channel. Whereas the TOM complex is thought to contain two to three pore-forming channels that mediate protein translocation, the TOC complex is thought to have four[59–63]. Determination of the pore sizes by electrophysiological measurements and electron microscopy of the channel protein Tom40, isolated from the yeast *Neurospora crassa* and from rats, as well as Toc75 isolated from peas, reflects the pore sizes found in purified complexes; thus these two proteins are considered to form pores that translocate precursor proteins[59–63,89–93]. The pores formed by Tom40 and Toc75 have a β-barrel structure, which forms a cation-selective channel of about 2.2–2.5 nm and 1.4–2.6 nm, respectively[59,89–93]. The pores are therefore of sufficient size to transport an unfolded polypeptide chain of the precursor protein into mitochondria or chloroplasts. The Tom40 pore can even accommodate precursor proteins such as the uncoupling protein (UCP) or the ATP/ADP carrier (AAC) in a α-helical hairpin structure[94,95]. However, given the low dynamic capability of the β-barrel structure in the pore, there is insufficient capacity for the translocation of folded proteins.

Although they have similar globular properties, Tom40 and Toc75 do not belong to the same protein family. Tom40 is assumed to have evolved from the same bacterial ancestor as the mitochondrial porin VDAC[96,97]. By contrast, Toc75 belongs to the highly conserved family of Omp85 homologues that assemble bacterial outer-membrane proteins[98,99]. Interestingly, another member of this protein family, Sam50, also localizes to the mitochondrial membrane to mediate the insertion of β-barrel outer-membrane proteins[96,99] (BOX 1; FIG. 1a). Similarly, in chloroplasts there is a second essential Toc75 homologue, Toc75-V (also known as OEP80), that is not part of the TOC translocon[100] (BOX1; FIG. 1b). One can speculate that Toc75-V might be the chloroplast counterpart of the mitochondrial Sam50, as it is essential for plant development[101].

Structural analysis of the Omp85 protein family has revealed two distinct domains: a soluble domain at the N terminus with different numbers of repeats annotated as polypeptide-transport-associated (POTRA) domains[102–104], and a pore-forming β-barrel domain composed of 16 β-strands in the carboxy-terminal portion of the protein[105]. The most C-terminal POTRA domain contains a loop that classifies the different Omp85 proteins[104], but its functional relevance remains to be established. In the case of Toc75, the POTRA domain acts as a specific low-affinity receptor site for precursor proteins containing a targeting sequence. This domain further interacts with Toc34 (REF. 106). At present, however, it remains unknown whether the Toc34 interaction is required for the import of Toc75 or whether it has a physiological role in the assembled complex. In mitochondria, direct interactions of Tom40 with substrates and other TOM subunits, such as the small Tom proteins, have been described[107–109], but it is not known through which domains this occurs.

Interestingly, in mitochondria, Tom22 can modulate the channel opening of Tom40 (REFS 64,93). It seems possible that Toc159 also closely cooperates with the translocation pore for two reasons. First, Toc159 contains a large protease-protected domain that faces the intermembrane space[65]. The function of this domain remains enigmatic, but might be related to the activity of Toc75. Second, Toc159 and Toc75 are sufficient for translocation of a precursor protein across a membrane, and Toc159 has been suggested to localize in the centre of the TOC complex[110]. Although this requires further experimental support, it is possible that the central translocon organizer Tom22 or Toc159 is also important for pore regulation.

The driving force of precursor protein translocation. Although the mitochondrial and chloroplast translocation machineries are comparable in their overall composition, the ways in which they recognize and deliver precursor proteins are remarkably different (FIG. 3c). For mitochondria, an 'affinity chain' hypothesis is proposed, in which precursor proteins show increasing affinity for receptors in the order of their recognition[111]. By contrast, import through the TOC complex is thought to depend on GTP binding and hydrolysis.

ERMES complex
(Endoplasmic reticulum–mitochondria encounter structure complex). This is the complex that tethers mitochondria and the endoplasmic reticulum. It is composed of the two mitochondrial membrane proteins Mdm34 and Mdm10, the integral endoplasmic reticulum membrane protein Mmm1 and the peripheral protein Mdm12.

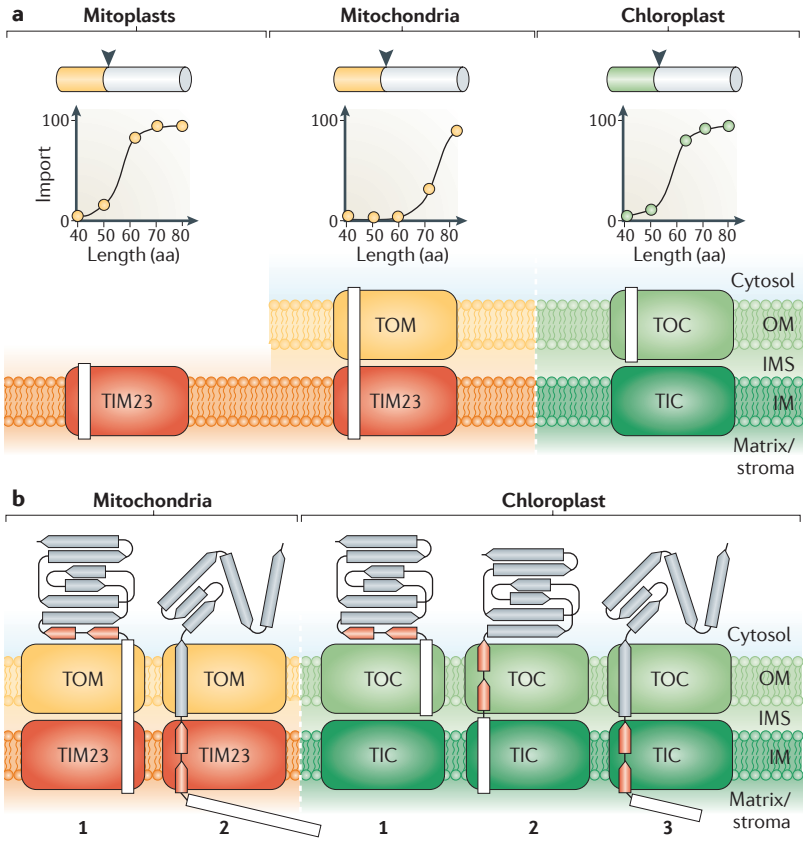


Figure 4 | **Signal length requirement and translocon initiation. a** | The translocation of precursor proteins (shown as cylinders at the top) across the membrane requires the signal peptide to be a certain amino acid (aa) length. The efficiency of *in vitro* import of precursor proteins with signals of variable length (but always with the same passenger domain) into the matrix of mitoplasts (mitochondria with removed outer membrane) or mitochondria, or the stroma of chloroplasts has been quantified[120–123]. The efficiency of import in relation to the signal length is presented schematically; dots are included only for better visibility and do not reflect experimental values. Precursors are translocated into mitoplasts by the translocase of the inner membrane of mitochondria 23 (TIM23) machinery alone (BOX1; FIG. 1). For translocation into mitochondria, the signal peptide (white cylinder) has to pass through both the translocase of the outer membrane of mitochondria (TOM) complex and the TIM23 machinery. The length required for translocation into chloroplasts is similar to that for mitoplasts, suggesting that the signal only has to pass through the translocase of the outer membrane of chloroplasts (TOC) complex machinery, and not into the translocase of the inner envelope membrane of mitochondria (TIC) complex machinery (BOX1; FIG. 1), to enable successful translocation into the stroma. **b** | The initiation of translocation of titin as an artificial passenger protein fused to a signal peptide requires distinct energizing events[123–126]. In each case, a signal peptide of distinct length was used. Mitochondrial translocation is energized by two events: the signal peptide engages the inner-membrane-associated mitochondrial motor (**1**), which leads to unfolding and translocation of the passenger (**2**). Translocation into chloroplasts involves three distinct events. An early event, possibly at the TOC complex, leads to rapid detachment of the amino-terminal region (red) from the globular fold (grey) (**1**), resulting in the transfer of the signal peptide across theTIC complex, leading to the interaction with stromal chaperones[139–141] (**2**). The latter interaction is required for the final unfolding and translocation events (**3**). IM, inner membrane; IMS, intermembrane space; OM, outer membrane.

On the cytosolic face of the mitochondrial membrane, the presequences of precursor proteins are recognized by a hydrophobic cleft in the cytosolic domain of Tom20 (REF. 85), and by a negatively charged cytosolic domain of Tom22 (REF. 112). Subsequently, the precursor is transferred to and through the Tom40 pore (FIG. 3c). Tight coupling to downstream translocation events in the intermembrane space or in the inner membrane provides the energy needed to complete import across the mitochondrial outer membrane[14,15].

In chloroplasts, transfer is regulated differently. The two chloroplast receptors Toc159 and Toc34 are GTPases[47,48,82], whereas Tom22 and Tom20 act independently of nucleotides (FIG. 3c). Precursor-protein recognition by Toc34 and Toc159 is nucleotide-dependent, although the exact mode remains a matter of debate[65,113–115]. In a minimal, reconstituted complex in liposomes, Toc159 and Toc75 (but not Toc75 alone or in combination with Toc34) can transfer a precursor protein across the membrane in a manner dependent on GTP hydrolysis[110]. On the basis of this, it was suggested that Toc159 provides the force for initial precursor-protein translocation. However, recent observations challenge this model: Toc159 mutants partially lacking the GTPase domain[116], and mutants of Toc159 with reduced intrinsic GTPase activities, almost completely rescue the *toc159* knockout[117,118]. As a result, whether GTP hydrolysis is required for precursor translocation, or whether Toc159 simply regulates channel opening through its membrane-embedded domain, remains unsolved. Nevertheless, the 'charging' of precursor proteins to be translocated by Toc159 together with the action of Hsp70 localized in the intermembrane space[119] might provide the energy to complete transfer through the outer envelope membrane. Hsp70 forms a complex with Toc64 and the J-domain protein Toc12 (FIG. 3c) that can activate the function of Hsp70-type chaperones *in vitro*[119]. Together, these studies suggest that distinct processes drive translocation into chloroplasts and mitochondria.

Comparisons of the polypeptide lengths required for translocation and of the capacity to translocate passengers with different unfolding properties provide additional insight into how translocation through the mitochondrial TOM complex occurs compared with that of the chloroplast TOC complex. First, for efficient import of a folded passenger protein into mitochondria, a signal peptide of at least 75–80 amino acids is required[120,121] (FIG. 4a). After rupture of the outer mitochondrial membrane to form mitoplasts, the precursor protein can directly engage the presequence translocase (TIM23 complex) of the inner membrane (BOX 1). Under these conditions, a signal peptide of about 60 amino acids is sufficient for translocation of precursor proteins across the mitochondrial inner membrane[120] (FIG. 4a). These data indicate that the signal length requirement correlates with the distance between the precursor entry point and the first binding site of an 'energizing unit' present in the mitochondrial matrix. Interestingly, a signal of 60 amino acids is sufficient for translocation of a folded precursor protein into chloroplasts[122,123], which is very similar to the length required for translocation across the inner membrane of mitochondria. On the basis of this observation, it can be envisioned that the chloroplast signal peptide has to be transferred across just one membrane to engage an energizing unit, whereas the mitochondrial signal peptide has to be transferred across two membranes simultaneously to provide the energy needed for translocation.

Table 2 | **The global elements of protein translocation**

Element	Mitochondria	Chloroplasts
Targeting signals	• Cleavable presequence • Non-cleavable internal signal • Cleavable presequence followed by hydrophobic sorting signal for intermembrane space proteins (bipartite signal)	• Cleavable transit peptide • Non-cleavable internal signal • Cleavable targeting signal followed by sorting signal for thylakoid proteins (bipartite signal)
Main targeting factors	Hsp70/Hsp90, Msf	Hsp70/Hsp90, 14-3-3
Receptor platform	Tom20, Tom22, Tom70–TPR domain	Toc34, Toc159, Toc64–TPR domain
Pore-forming protein	Tom40, β-barrel, porin-like	Toc75, β-barrel, Omp85
Pore size	2.2–2.5 nm	1.4–2.6 nm
Pore number*	2–3	4
Complex integrity	Tom22, Tom20, Tom6, Tom7	Toc159, Toc34
Driving force	Affinity driven	Nucleotide dependent
Regulation	-	GTP binding and hydrolysis phosphorylation
IMS-sorting platform‡	Tom22, Tom7, Tom40	Toc159, Toc64, Tic22, Toc12, Hsp70
Supercomplexes	TOM–TIM23 translocases	TOC–TIC translocases

*Pore number observed within the complex. ‡Intermembrane space (IMS)-localized sorting platform of the translocase of the outer membrane of mitochondria (TOM) and translocase of the outer membrane of chloroplasts (TOC) complexes. Hsp, heat-shock protein; Msf, mitochondrial import-stimulating factor; TPR, tetratricopeptide repeat.

Second, the transfer kinetics of an artificial precursor protein across the mitochondrial and chloroplast membranes have been measured to explore the force and energetics provided by the translocon. For this purpose, the 27th domain of the human muscle protein titin (I27) was fused to an organelle-specific presequence[124,125]. The titin domain is folded in the presence of the presequence (FIG. 4b, white bar) and contains an N-terminal strand (red) that is followed by a stable globular domain (grey). The titin domain has to be unfolded during translocation into an organelle and it has been shown, using mutants that affect the stability of titin, that the rate of import into mitochondria correlates with the unfolding of the entire titin molecule and that mutations in the N-terminal strand behave similarly to those in the globular domain[124,125]. This observation is consistent with the idea that a single source provides the force needed for unfolding the precursor protein. By contrast, the translocation rate across the envelope membranes of chloroplasts is affected only by mutations in titin's globular domain, and not by mutations in the N-terminal strand[126]. Thus, in the case of chloroplasts, initial unfolding of the titin N-terminal strand follows a different energetic regime from final translocation.

Taken together, it has to be suggested that, in contrast to mitochondria, the force for translocation into chloroplasts is provided by machinery that is engaged after precursor proteins cross the first membrane (step 1). The existence of this 'energizing unit' leads to the initial unfolding of titin required for the translocation across the inner membrane. Finally, and comparable to the mitochondrial system, an energy-providing unit in the stroma promotes final translocation (step 2).

Regulation of transport across the outer membrane by post-translational modifications of complex components has only been reported for the TOC translocon. Phosphorylation of Toc34 inhibits its GTP-binding and so precursor recognition *in vitro*[127], and also disturbs complex formation[128]. Similarly, the acidic N-terminal region of Toc159 can be phosphorylated *in vitro*[127,128]. So far, however, it is not understood under which conditions this mode of regulation affects protein translocation by either of these two receptors *in vivo*.

Translocase regulation of sorting

After transfer across the outer membrane, precursor proteins have to be sorted to different subcompartments (BOX 1). In both mitochondria and chloroplasts, the translocon of the outer membrane provides a binding site in the intermembrane space. In mitochondria, Tom40, Tom7 and Tom22 bind to the precursor protein on the intermembrane-space side of the membrane and might facilitate the first sorting steps[129–131]. In chloroplasts, Toc64, Toc12, Tic22 and Hsp70 are thought to interact with incoming precursor proteins in the intermembrane space[74,119]. Crosslinking experiments indicate that Toc159 also comprises a binding site for precursor proteins on the intermembrane-space side of the outer membrane[65,113]. However, unlike in mitochondria, sorting in the intermembrane space of chloroplasts is not yet understood.

In mitochondria, the intermembrane-space domain of Tom22 might ensure precursor-protein transfer to the presequence translocase of the inner membrane (TIM23 complex)[132]. In chloroplasts, chemical crosslinking can also occur between Toc159 and precursor proteins arrested in a TOC–TIC supercomplex[113] and there are some suggestions that Tic22 might link the translocases of the outer and inner envelope membranes[113,119]. Thus, in both mitochondria and chloroplasts the two translocases of the outer and inner membranes can closely coordinate efficient transport of precursors across the intermembrane space. This view is supported by the observation of a supercomplex of the two complexes in both organelles after arrest of precursor-protein transfer[132–135].

Conclusions

Protein targeting and transport through the outer membrane translocase of mitochondria and chloroplasts share several features (TABLE 2). Both translocases are equipped with a set of receptor proteins that bind to cleavable and non-cleavable substrates. Each complex consists of a functional core module with a loosely associated receptor protein — Tom70 in the case of mitochondria and Toc64 in chloroplasts — that recognizes cytosolic chaperones such as Hsp70 or Hsp90 through a TPR domain (TABLE 2). The receptors Tom20 and Toc34 are crucial for the import of most presequence-containing precursor proteins, and their binding to substrates occurs in cooperation with Tom22 and Toc159,

Chemical crosslinking
Chemical crosslinking is the introduction of synthetic bonds that link two proteins in close proximity by chemical molecules — for example, by maleimide, which reacts with the thiol group of cysteines.

respectively. Tom22 and Toc159 not only transfer the precursor proteins towards the translocation channel that is formed by a β-barrel protein, but also mediate docking of other receptors. In addition, Tom22 regulates channel opening and mediates precursor-protein transfer to the TIM23 translocase through its intermembrane-space domain. It is not yet clear whether Toc159 carries out similar functions in chloroplasts. Taken together, we propose that Tom22 and Toc159 are central organizers of the outer-membrane translocase in mitochondria and chloroplasts.

These similarities are remarkable given that the core receptor proteins of the TOM and TOC translocons have such distinct molecular features. Toc159 and Toc34 are both GTPases, and therefore transport into chloroplasts is tightly coupled to GTP binding and hydrolysis. Moreover, phosphorylation of both Toc receptors modulates precursor-protein recognition, and in the case of Toc34, complex association. By contrast, Tom20 and Tom22 act independently of nucleotides, and increasing affinity drives the transport of precursor proteins across the mitochondrial outer membrane; it is not clear whether this process is regulated or constantly active.

The structure of the translocating unit has not yet been determined for either system, and should give important insight into how a β-barrel channel can accommodate precursor protein and also interact with receptor proteins that have α-helical transmembrane domains. This should also help us to understand the regulation and mechanism of precursor-protein translocation across the outer membrane of these two cell organelles of endosymbiotic origin.

The global elements defined for the TOM and TOC transport systems — including targeting signals and factors, receptor proteins, a translocation pore and a driving force for import — are shared by other translocases. Protein transport of this type occurs in peroxisomes, the ER and at the bacterial plasma membrane. However, the structural components fulfilling these tasks differ remarkably. The TOC and TOM translocases are the only transport machines in which precursor proteins pass through a β-barrel pore. β-barrel proteins are also involved in the insertion of proteins into the outer membrane of mitochondria and bacteria, but there is no evidence that precursor proteins are transported through the β-barrel for membrane integration[4,96,102,105]. Whether the TOM and TOC translocons are also directly involved in the insertion of outer-membrane proteins (such as those with α-helical membrane regions) is currently not clear. If they are involved, this process would not involve lateral diffusion of the membrane domain out of the β-barrel for energetic reasons. By contrast, α-helical translocating channels allow the lateral release of precursor proteins into the bacterial plasma membrane, the microsomal membrane and the inner mitochondrial membrane[9,11,14,24]. In addition, although different receptors for specific targeting pathways can also be found in transport systems such as those of peroxisomes, the presence of a multifunctional receptor unit is a characteristic feature of the translocation systems of endosymbiotically derived organelles[11]. The TOM and TOC complexes are also unique in that they are the only complexes that interact with another membrane-embedded translocon, because mitochondria and plastids are the only two organelles surrounded by at least two membranes. Future work has to decipher the structural and functional details of the communication between the two complexes in the outer membrane and the other translocons. This may have implications for the cooperation of protein machineries in general.

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