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Review

Protein translocation across membranes

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Abstract

Cellular membranes act as semipermeable barriers to ions and macromolecules. Specialized mechanisms of transport of proteins across membranes have been developed during evolution. There are common mechanistic themes among protein translocation systems in bacteria and in eukaryotic cells. Here we review current understanding of mechanisms of protein transport across the bacterial plasma membrane as well as across several organelle membranes of yeast and mammalian cells. We consider a variety of organelles including the endoplasmic reticulum, outer and inner membranes of mitochondria, outer, inner, and thylakoid membranes of chloroplasts, peroxisomes, and lysosomes. Several common principles are evident: (a) multiple pathways of protein translocation across membranes exist, (b) molecular chaperones are required in the cytosol, inside the organelle, and often within the organelle membrane, (c) ATP and/or GTP hydrolysis is required, (d) a proton-motive force across the membrane is often required, and (e) protein translocation occurs through gated, aqueous channels. There are exceptions to each of these common principles indicating that our knowledge of how proteins translocate across membranes is not yet complete. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Protein; Translocation; Membranes

1. Introduction

Proteins are able to translocate across membranes in a molecule-by-molecule fashion by mechanisms that share several common features, and such translocation pathways are the subject of this review. Packets of proteins can also cross membranes by the fusion of protein-filled vesicles with recipient

Abbreviations: AAC, ATP/ADP carrier; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; cHsp70, chloroplast Hsp70; DHFR, dihydrofolate reductase; DnaJ, a bacterial gene encoding an hsp70 cochaperone; ER, endoplasmic reticulum; ffh, fifty-four (kDa) homologue; GIP, general import pore; GRP78, glucose-regulated protein of 78 kDa; Hsc70, heat shock cognate protein of 70 kDa; Hsp70, heat shock protein of 70 kDa; Hsp40, heat shock protein of 40 kDa; Lamp2a, lysosome-associated membrane protein 2a; Lyhsc70, lysosomal hsc70; Mge1p, a yeast cochaperone for mHsp70; MPP, matrix processing peptidase; MSF, mitochondrial stimulating factor; mtHsp70, mitochondrial Hsp70; PTS, peroxisome targeting sequence; Sec, secretory; SPase, signal peptidase; SRP, signal recognition particle; SR, signal recognition particle receptor; TAT, twin arginine translocation; Tic, translocon of the inner chloroplast membrane; Tim, transporter of the inner mitochondrial membrane; Toc, translocon of the outer chloroplast membrane; Tom, transporter of the outer mitochondrial membrane; TRAM, translocating chain-associated membrane

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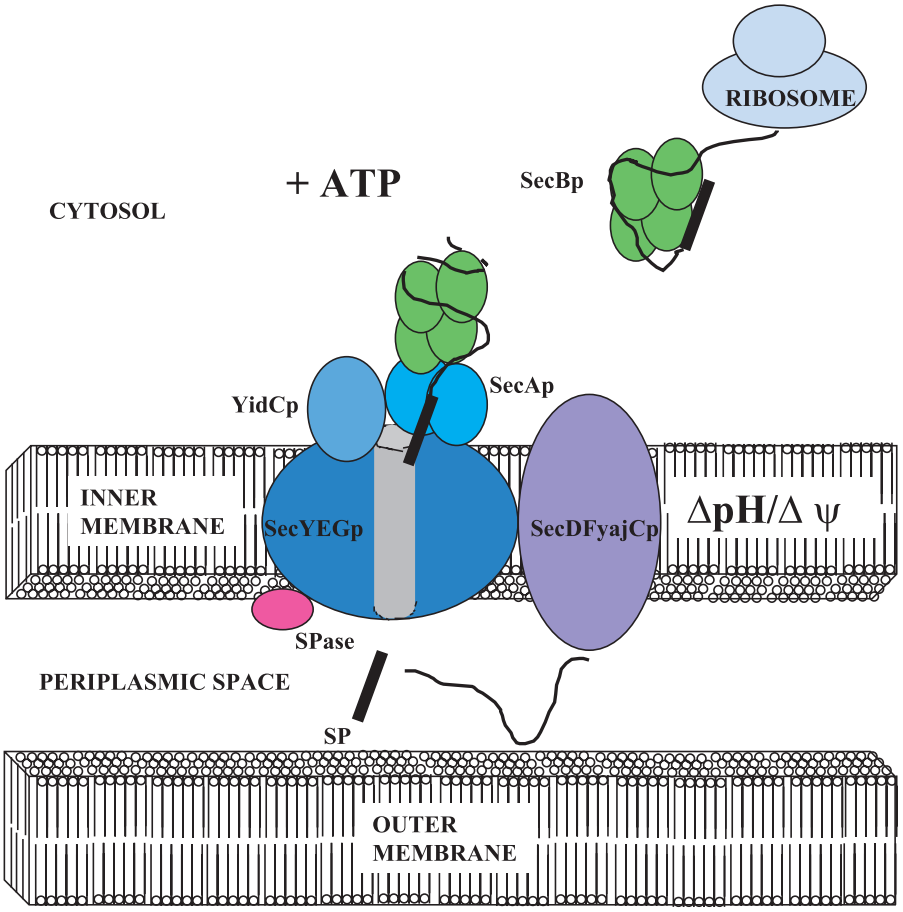

Fig. 1. The Sec pathway of bacteria. The Sec proteins are designated Ap, Bp, etc. The protein components in the inner membrane that are required for the operation of the Sec pathway are shown. ΔpH/ΔΨ, proton-motive force; SP, signal peptide; SPase, signal peptidase.

membranes. These vesicular pathways are important for protein secretion, endocytosis, and targeting of some proteins to their appropriate organelles. These areas have been the subjects of many recent reviews [1–5]. In addition, vesicular mechanisms are responsible for the delivery of proteins to lysosomes by macroautophagy. Understanding of this process has dramatically advanced and has been the subject of recent reviews [6,7]. These vesicular pathways are not included in this review.

2. Protein translocation across the bacterial plasma membrane

At least four protein translocation/secretion pathways have been described in Gram-negative bacteria such as *Escherichia coli* [8]. The general translocation system is mediated by two molecular chaperones, SecAp and SecBp, and is therefore designated the Sec-dependent pathway. The twin arginine translocation (TAT) system does not require either Sec protein and is therefore known as the Sec-independent pathway. The TAT system is capable of translocation of metalloproteins and protein complexes in a folded state. In addition, two other pathways have been described for targeting of integral membrane proteins to the inner membrane, the signal recognition particle (SRP) system and the YidC-dependent pathway [9]. The SRP system is responsible for targeting and co-translational insertion into the plasma membrane of inner membrane proteins [8]. The SRP pathway in bacteria presents remarkable similarities to the co-translational translocation pathway in the endoplasm reticulum (ER) of eukaryotic cells [10]. The YidC-dependent pathway is required for the insertion of

some proteins, such as the coat protein of the phage M13, into the plasma membrane [9]. Whether or not other proteins can insert into membranes without the aid of membrane proteins, as was once thought for the M13 coat protein, remains to be seen.

2.1. The Sec-dependent pathway

The Sec pathway is responsible for the export of many newly synthesized outer membrane and periplasmic proteins across the plasma membrane [10] (Fig. 1). Proteins transported by this pathway have in common a signal peptide of 18–26 amino acids in their amino-terminal region [11–13]. These signal peptides have three characteristic regions: a positively charged amino acid at the N-terminus, a highly hydrophobic region, and a polar region containing the signal peptidase cleavage site [14]. The Sec translocon, defined as the minimal machinery that can accomplish protein transport across the membrane, can only export unfolded polypeptides [15]. Protein translocation by the Sec pathway is dependent upon both ATP and a proton-motive force [16–18]. The proton-motive force results from a combination of membrane potential ($\Delta\Psi$) and a pH gradient (ΔpH) [19]. Transport by the Sec pathway is inhibited by either sodium azide, an inhibitor of ATP production and of the activity of SecAp [20], or the protonophore reagent, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP).

SecAp is part of a multisubunit preprotein translocase complex in the plasma membrane together with SecYp, SecEp, SecGp, SecDp, SecFp, and SecyajCp [8,21] (Fig. 1). These integral membrane proteins form two distinct trimeric complexes: SecYEGp and SecDFyajCp. SecAp binds with high affinity to SecYEGp [22]. Several laboratories have shown that SecAp and SecYEp constitute the minimal requirement for preprotein translocation [23,24]. However, SecGp and SecDFyajCp are required for maximal rates of protein secretion [25,26]. YidCp also associates with the SecYEGp complex [27] (Fig. 1).

Electron microscopic studies of the purified active translocation SecYEp complex show that it forms a ring-like structure with tetrameric [28] or pentameric symmetry [29]. Based on the estimated molecular mass of 230 kDa, each ring would be formed by three to four SecYEp complexes. These ring-like structures may represent protein-conductive channels. The other components of the translocon, SecGp and SecDFyajCp, together with SecAp, promote the translocation of preproteins at the SecYEp core of the translocon (Fig. 1) [20,21]. Interestingly, both SecYp and SecEp have homology to components of the eukaryotic protein translocon of the endoplasmic reticulum [30]. SecYp is homologous to Sec61p in yeast and Sec61α in mammals [31–33]. SecEp has homology to Sss1p in yeast and Sec61γ in mammals [34].

The importance of membrane lipids in protein translocation processes is becoming increasingly apparent [1,35,36], and a phospholipid bilayer is depicted in Fig. 1. However, the details of lipid composition and organization surrounding protein translocons are scant, so lipid constituents have been omitted from other figures for clarity.

The cytosolic molecular chaperone SecBp associates with a long polypeptide sequence, over 150 amino acids [37,38] of either a fully translated preprotein [39] or a nascent polypeptide [40]. The functional form of SecBp is a homotetramer [8,41] (Fig. 1). SecBp not only maintains the preprotein in a translocation-competent state but also targets the newly synthesized polypeptide to the plasma membrane [42]. Unlike many other molecular chaperones, SecBp association with and dissociation from polypeptides is ATP-independent [8].

The crystal structure of the SecBp tetramer shows two long channels along the sides of the molecule that would be suited for binding a variety of polypeptides [43]. The SecBp–preprotein complex binds to a membrane-associated SecAp homodimer [44] (Fig. 1). SecAp can be associated with the plasma membrane by interacting with acidic lipids [45–47] but it is also present in the cytosol, and it is possible that SecAp binds the SecBp–preprotein complex first while in the cytosol [8]. Upon binding to SecAp, the preprotein promotes ATP binding to SecAp. ATP binding to SecAp induces a conformational change, and a portion of SecAp inserts into the plasma membrane probably through the SecYEp translocon core [48,49] along with 20–30 amino acids of the preprotein [18,50].

The preprotein also activates the ATPase activity of SecAp [19], and hydrolysis of ATP causes both the dissociation of the preprotein from SecAp and SecAp

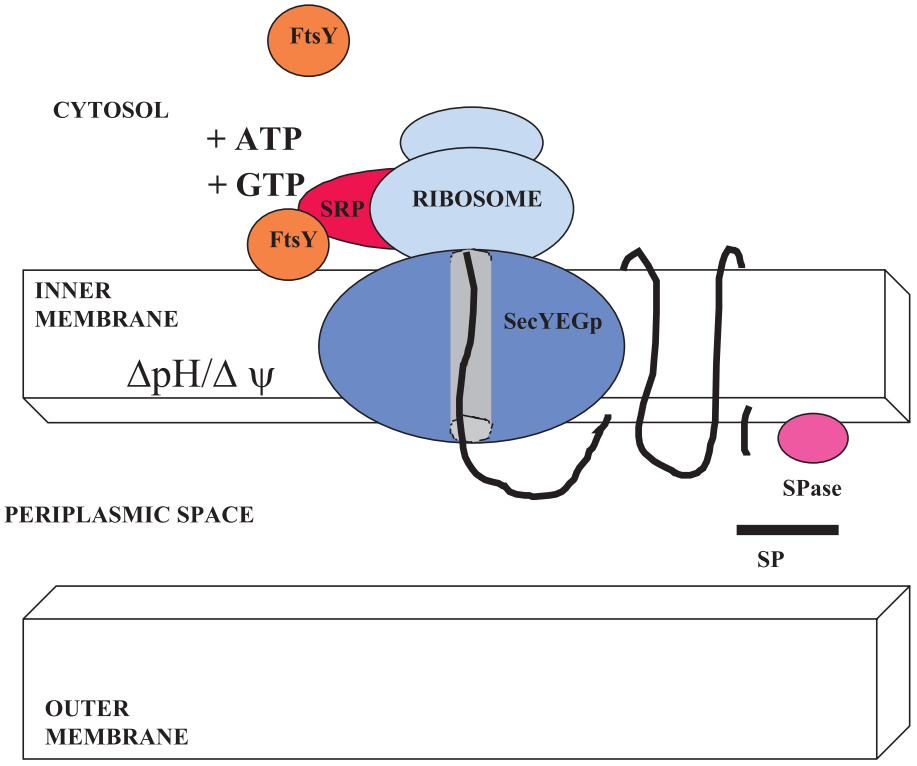

Fig. 2. The signal recognition particle protein translocation pathway in bacteria. This pathway uses the SecYEGp translocon as does the posttranslational Sec pathway. Peptide bond elongation by the ribosome, together with GTP hydrolysis, pushes the protein through the translocon, and protein often integrates into the inner membrane through this pathway. SRP, signal recognition particle; ΔpH/ΔΨ, proton-motive force; SP, signal peptide; SPase, signal peptidase.

deinsertion from the plasma membrane [51]. SecAp dissociates from the membrane and is exchanged by another SecAp dimer from the cytosol that results in the translocation of another 20–30 amino acids of the preprotein [51]. The proton-motive force across the membrane promotes further translocation of the polypeptide [18,22]. Several cycles of ATP binding and hydrolysis are required for successful translocation of a single polypeptide [52].

2.2. The signal recognition particle pathway

The mechanism of cotranslational targeting of precursor proteins to cellular membranes has been conserved during evolution from bacteria to eukaryotic cells. The SRP pathway in bacteria is involved primarily in translocation of inner membrane proteins [53] (Fig. 2). In addition, a small number of proteins are secreted by this pathway [54]. A particular feature of this pathway is that insertion into the translocon takes place cotranslationally. The bacterial SRP might act as a specific chaperone for highly hydrophobic signal sequences of nascent polypeptides [55]. This interaction might maintain the nascent polypeptide in a transport-competent conformation.

The bacterial SRP is a cytoplasmic ribonucleoprotein complex that consists of a 48 kDa protein and a 4.5S RNA molecule. The 48 kDa protein has homology to the eukaryotic SRP54 kDa subunit, and it is therefore designated as fifty-four homologue (ffh) [56,57]. The interaction between ffh and the translating ribosome requires GTP [58] (Fig. 2). In addition, the RNA molecule is homologous to the eukaryotic SRP 7S RNA [59]. The bacterial protein FtsY was also identified by homology to the α-subunit of the eukaryotic SRP receptor (SRα), [56,57]. FtsY is localized both to the plasma membrane and to the cytosol [54] (Fig. 2). No homologous bacterial protein has been identified for the β-subunit (SRβ) of the eukaryotic SRP receptor [60], and such a homologue may be unnecessary since FtsY binds directly

to acidic phospholipids in the membrane [36]. The SRP is released at the plasma membrane from the translating ribosome by FtsY, and this reaction requires GTP [10]. The nascent polypeptide inserts into the SecYEG translocon [10,61]. The SRP and the Sec pathways therefore share the same translocon on the plasma membrane [61].

2.3. The twin arginine translocation system

Proteins exported by this pathway have an unusually long signal sequence of up to 48 amino acids. This signal sequence has an invariant twin arginine motif in its amino-terminal region followed by a short hydrophobic sequence and one or more basic amino acids. A consensus twin arginine targeting motif is serine-arginine-arginine-X-phenylalanine-leucine-lysine (SRRXFLK) in which X can be any amino acid [62]. The twin arginine motif constitutes a 'Sec avoidance' signal [63]. Replacement of the arginine-arginine motif by lysine residues, increasing the hydrophobicity of the middle region, or elimination of the basic amino acid in the carboxyl terminus renders this protein incapable of translocation by the TAT pathway [15]. Interestingly, any of these modifications in the signal peptide transforms the protein into a substrate for the Sec-dependent pathway [15].

Genes and proteins involved in this pathway are designated by a unified nomenclature as *tat* and Tat, respectively (Fig. 3). Protein translocation by this pathway requires a proton-motive force across the bacterial inner membrane [15].

An unusual aspect of the TAT pathway is that proteins can be translocated across the plasma membrane in a folded state [14] (Fig. 3), a feature shared with protein transport into peroxisomes in eukaryotic cells [64] and with one pathway of transport into the thylakoid lumen of chloroplasts in plants [65]. Many of the substrates known for this pathway are metalloenzymes that reside in the periplasmic space or are associated with the periplasmic face of

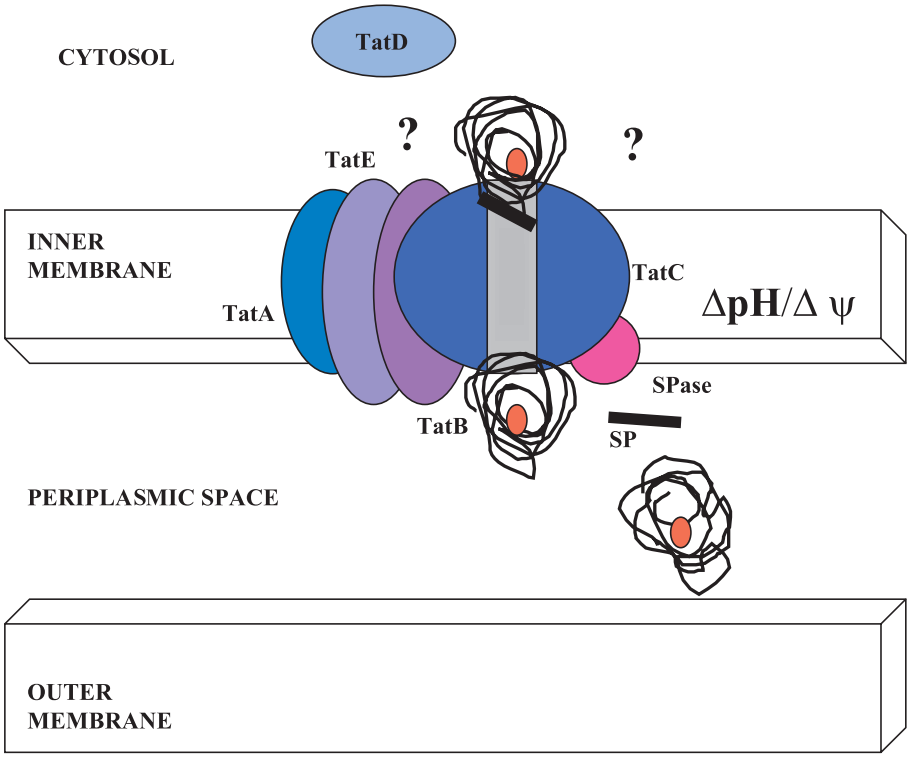

Fig. 3. The twin arginine protein translocation pathway in bacteria. TatA–E are proteins required for this pathway of protein translocation. $\Delta pH/\Delta\Psi$ indicates that a proton-motive force across the inner membrane is required for protein translocation. The protein shown is translocated in a folded state, and the small oval represents a cofactor that, when present, is bound to the protein prior to translocation. SP, signal peptide; SPase, signal peptidase.

the inner membrane [14]. Complex oxidation–reduction cofactors such as iron–sulfur clusters and nickel and iron cofactors [63] are acquired by the substrate proteins in the cytosol prior to translocation [66]. Further confirmation that proteins are transported in a folded state comes from the observation that the catalytic subunits of the periplasmic iron- and nickel-iron hydrogenases have neither Sec nor TAT signal sequences, while their regulatory subunits have TAT signal sequences [14]. The regulatory subunits are required for translocation of the catalytic subunits [67] indicating that these enzymes are likely to be translocated as a multimeric complex [14].

The TAT pathway is similar in both thylakoid protein translocation and in bacterial protein secretion [68–70]. HCF106 is a gene involved in a Sec-independent and proton-motive force-dependent import pathway in thylakoid transport in maize chloroplasts [71]. Several genes have been identified by homology to HCF106 in bacteria and in higher plants [71,72]. *E. coli* has three HCF106 homologues, *tatA*, *tatB*, and *tatC* [69,72,73]. In addition, *tatD* and *tatE* form part of the TAT system [72]. All Tat proteins are associated with the inner membrane of *E. coli*, with the exception of TatD which is a cytosolic protein as predicted by sequence analysis [72] (Fig. 3). TatA, TatB, and TatE have a single transmembrane domain, while TatC has been predicted to contain six transmembrane domains [72]. Genetic and biochemical analyses demonstrated that inactivation of TatC eliminates transport of all the protein substrates [63]. On the other hand, deletion of either *tatA* or *tatE* has a partial effect on transport, indicating that these proteins have overlapping functions [72]. Deletion of *tatB* results in a rapid degradation of TatC, indicating that TatB might interact directly with TatC and be important for its stabilization [74].

As mentioned earlier, the translocation of proteins by the TAT pathway is dependent on the proton-motive force across the membrane [75] (Fig. 3). Transport is inhibited by the protonophore reagent, CCCP, but not by sodium azide. In addition, depletion of intracellular ATP does not affect the transport of protein substrates [66]. TatC could form the channel or translocon, while TatA, TatE, and TatB might act as receptors for signal sequences of different substrates [71,73,75] (Fig. 3).

The translocation complex has to be able to allow the translocation of a folded protein and maintain the separation between the cytosol and the periplasmic space to preserve the proton gradient. It is possible to imagine the translocon as a 'zipper-like' or 'sphincter-like' structure where the periplasmic side is closed while the protein substrate is beginning to be translocated. In the middle of the transport process, the folded protein itself would act as a physical barrier. By the end of the process the cytosolic side of the translocon would be closed while the protein exits the translocon and a signal peptidase cleaves the signal peptide (Fig. 3). Much more information is needed to fully understand how folded proteins can translocate across membranes.

3. Eukaryotic protein translocation

Subcellular compartmentalization is a distinctive feature of eukaryotic organisms. As most of the protein content of the different organelles must be imported from the cytosol, eukaryotic cells have developed specific systems for: (1) recognition of newly synthesized polypeptides in the cytosol, (2) targeting of these proteins to their appropriate organelle, (3) recognition of the substrate proteins by surface components of the organelle, and (4) vectorial translocation of these proteins into or across the organelle membranes [76].

A combination of genetic and biochemical approaches have produced a large body of information about translocation of precursor proteins into the ER, mitochondria, and chloroplasts. These approaches have only recently been applied to the study of protein translocation into peroxisomes and lysosomes, so our knowledge of these organelles is less complete. Protein translocation into and out of the nucleus occur through distinct nuclear pore structures by mechanisms that seem quite different from other membrane translocation pathways. Therefore, nuclear transport is not included in this review, but interested readers can consult several recent reviews [77–79]

3.1. Endoplasmic reticulum protein import pathways

In eukaryotic cells the majority of polypeptides destined to cross the ER membrane are translated

and translocated simultaneously. In addition to resident ER proteins, many proteins destined for secretion or for residence in the plasma membrane, the Golgi apparatus, lysosomes, and the endosomal compartments also enter the ER lumen cotranslationally [80–82]. Proteins targeted to the ER are synthesized with a signal sequence usually in the amino-terminal region similar to the sequence described for bacterial proteins utilizing the Sec pathway [13,56,76,82–84] (Fig. 4). The SRP binds both to the signal sequence in the nascent polypeptide and the translating ribosome and targets the complex to the ER membrane [85] (Fig. 4). Binding of the SRP to the translating ribosome results in slowing of translation [86].

The signal recognition particle consists of a complex of six polypeptides and one molecule of RNA [85,87]. The 54 kDa subunit of the SRP is responsible for binding to the signal sequence, and this interaction increases the SRP's affinity for GTP [80,88]. The SRP binds to its receptor (SRαp/SRβp) on the surface of the ER, and the ribosome binds to the translocation site on the ER membrane [89]. The interaction between the SRP and SRαp induces the hydrolysis of GTP [81,90,91]. As a consequence, the SRP is released into the cytosol, the ribosome binds to the ER membrane, and the nascent polypeptide is transferred into the aqueous channel of the translocon [92,93].

The ribosome binds tightly to the Sec61p complex in the ER membrane [94,95]. The Sec61p complex consists of three polypeptides, Sec61αp, Sec61βp, and Sec61γp (Fig. 4). This protein complex is responsible for the formation of the aqueous channel and the initial recognition of the signal sequence during the insertion of the nascent polypeptide into the translocon [95]. Interestingly, Sec61αp and Sec61γp are the eukaryotic homologues of bacterial SecYp and SecEp, respectively [31,32,34]. The translocating chain-associated membrane (TRAM) protein preferentially interacts with the signal sequence of most of the proteins translocated during the early stages of protein translocation [96] but its exact function in protein translocation is not clear. The SR, the Sec61p complex, and the TRAM protein constitute the minimal requirement for reconstitution of protein translocation in liposomes [96].

The glucose-regulated protein of 78 kDa (GRP78) is a member of the heat shock protein of 70 kDa (Hsp70) family, a molecular chaperone that is localized to the ER lumen. This molecular chaperone and

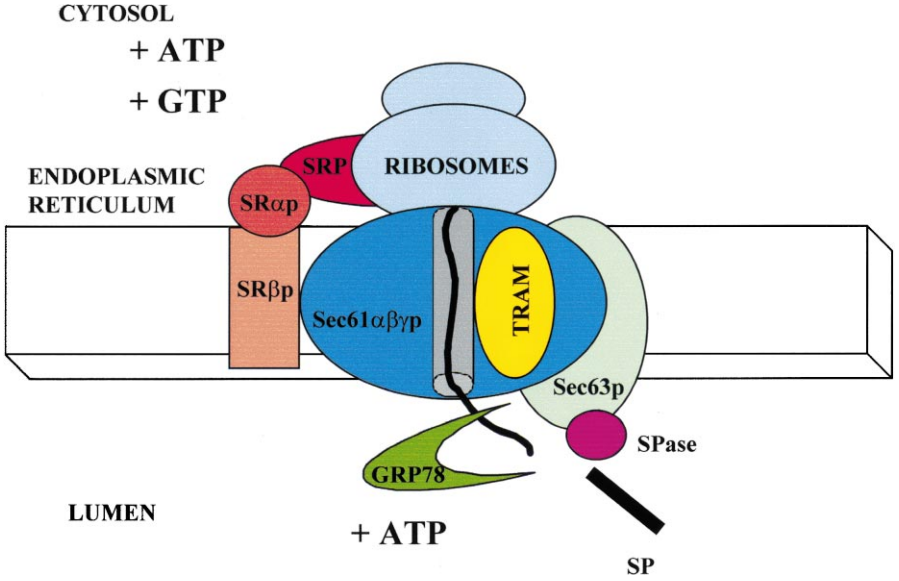

Fig. 4. The signal recognition particle pathway of protein translocation into the ER. The signal recognition particle (SRP) binds to its receptor (SR) in the ER membrane as well as to the docked ribosome. The translocon consists of the Sec61α,β,γ proteins, TRAM, and Sec63p. The translocation requires GTP hydrolysis (+GTP). The protein synthetic machinery provides part of the driving force to translocate the growing polypeptide chain across the membrane, and protein synthesis requires both GTP and ATP. The Hsp70 GRP78 in the ER lumen is required for the substrate to enter the ER lumen. The signal peptidase (SPase) cleaves the hydrophobic signal peptide (SP) after the protein enters the ER lumen.

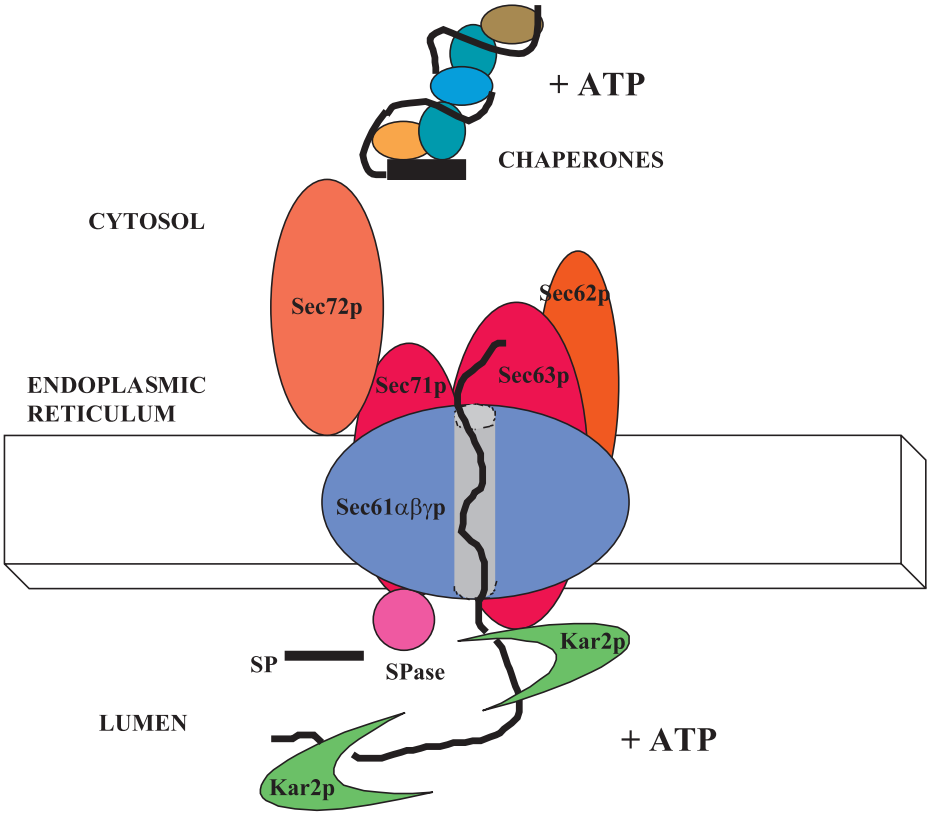

Fig. 5. Posttranslational translocation of proteins into the ER. A cytosolic complex of molecular chaperones and ATP (+ATP) facilitate this pathway of protein translocation. The translocon consists of the Sec61α,β,γ proteins and Sec63p. Other proteins, Sec62p, Sec71p, and Sec72p, are also required for efficient protein translocation. Kar2p is the yeast homologue of GRP78 and is required to pull the substrate protein into the ER lumen concomitant with ATP hydrolysis (+ATP). The signal peptide (SP) at the amino terminus of substrate proteins is cleaved by signal peptidase (SPase).

its yeast homologue, Kar2p, are required for the complete transport of proteins into the ER lumen [76,97,98] (Fig. 4).

All eukaryotic cells also have an SRP-independent pathway where fully translated ER precursor proteins are targeted to and translocated across the ER membrane [82,89,96]. Little is known of this pathway in higher eukaryotes [99]. On the other hand, studies in yeast have shown the translocation of large precursors posttranslationally, and that both targeting pathways function in parallel [83]. The hydrophobicity of the signal sequence of the ER precursor may determine whether a polypeptide is targeted by the cotranslational or the posttranslational pathway [83]. Precursor proteins with very hydrophobic signal sequences are preferentially recognized by the SRP, while less hydrophobic signal sequences could follow either pathway [83].

The *Saccharomyces cerevisiae* posttranslational translocon, like the cotranslational translocon, is formed by seven polypeptides containing the Sec61p complex and the Sec62–Sec63 complex (Fig. 5). The Sec62–Sec63 complex contains four polypeptides: Sec62p, Sec63p, Sec71p and Sec72p. Higher eukaryotic homologues of Sec62p and Sec63p have been identified [100]. Cytosolic Hsp70s stimulate the import of proteins into the ER [101] (Fig. 5). Cytosolic Hsp70s are associated with a variety of cochaperones that regulate their activities (Fig. 5) [102,103]. The ER lumenal Kar2p, like other Hsp70s, is an ATPase that binds transiently to the cochaperone DnaJ-like domain on the lumenal side of Sec63p [104]. Kar2p is required for the complete translocation of substrates into the ER lumen by both the posttranslational and the cotranslational translocation pathways [76,97] (Fig. 5).

Rapoport and colleagues have reproduced the translocation process with purified translocon complexes in a detergent solution in the absence of membranes [98]. The translocation reaction requires an intact DnaJ-like domain on Sec63p, ATP, and Kar2p/GRP78. The authors proposed that protein translocation is driven by Brownian movement, and that transient binding of Kar2p/GRP78 to the substrate prevents its backward movement. In addition, Kar2p was shown to be responsible for sealing the translocon after completion of translocation [105].

The transmembrane protein Sec71p together with the peripheral membrane protein Sec72p have been identified as proteins associated with the Sec62–Sec63 complex [106,107]. Sec72p is absent from the ER membrane when the gene *SEC71* is deleted, indicating that the proteins may interact with each other [108] (Fig. 5). Their function remains to be determined. No homologous proteins have been identified in higher eukaryotes to date [82].

3.2. Mitochondrial protein translocation

Although mitochondria contain their own genome, most mitochondrial proteins are encoded by nuclear genes and are synthesized on free ribosomes in the cytosol as precursors. Mitochondrial biogenesis requires protein targeting to four compartments: the outer membrane, the intermembrane space, the inner membrane, and the matrix (Fig. 6).

Translocation of mitochondrial precursors is an energy-dependent process that is assisted by heteromeric translocation complexes in both membranes. Four translocation complexes have been identified, two translocons in the outer mitochondrial membrane (Tom) and two in the inner mitochondrial membrane (Tim) (Fig. 6). Many matrix proteins cross the outer and inner mitochondrial membranes at locations of close contact between the two membranes. Interestingly, Tim23 spans both the inner and the outer membranes [109] likely at contact sites. In addition, some proteins can be imported into mitochondria independently of these translocation complexes perhaps due to their abilities to spontaneously associate with membrane bilayers or to associate with the translocon without the aid of receptors [110].

Precursor proteins destined to the mitochondrial matrix are usually hydrophilic polypeptides with an amino-terminal signal peptide that forms amphipathic helices in solution [11,111]. On the other hand, inner membrane proteins, such as the ATP/ADP carrier (AAC) and the phosphate carrier, are hydrophobic and contain internal targeting signals yet to be determined [112]. Both types of precursor proteins share the general import pore (GIP) for transport across the mitochondrial outer membrane, but they bind to a distinct set of receptors [113]. The GIP complex is defined by the pore-forming protein Tom40 and the small integral membrane proteins Tom5, Tom6, and Tom7 [112, 114,115] (Fig. 6).

Mitochondrial precursor proteins are imported in an unfolded state [116,117]. Cytosolic molecular chaperones such as Hsp70s and the mitochondrial stimulating factor (MSF) maintain the newly synthesized precursor proteins in a transport-competent state and target them to mitochondria [118,119]. MSF is an ATP-dependent cytosolic chaperone that interacts preferentially with certain mitochondrial precursors [118,119]. Other cytosolic chaperones are likely to be involved in this translocation pathway since mitochondrial precursor proteins exist as high molecular weight complexes [120,121].

Matrix precursors are recognized by the membrane receptors Tom20 and Tom22 [115], the cytosolic domains of which are capable of recognizing different characteristics of the signal peptide [122]. After binding to the receptors, the precursor protein interacts with Tom5, a protein thought to be involved in transferring the precursor from the receptors to Tom40, the main component of the translocation channel [123]. Tom40, similarly to bacterial porins, is composed of a series of β-sheets that form a β-barrel [124]. An at least partially functional translocon was reconstituted in vitro with purified Tom40 alone [125].

The transition from receptor-bound state to insertion into the GIP is ATP-dependent [126,127] (Fig. 6). A 400 kDa complex composed of Tom22, Tom40, Tom5, Tom6, and Tom7 was purified from yeast mitochondria, along with a 170 kDa complex of Tom70 and Tom37 [121,128]. Tom6 regulates the activity of Tom22 [128–130]. The driving force for protein translocation across the outer membrane

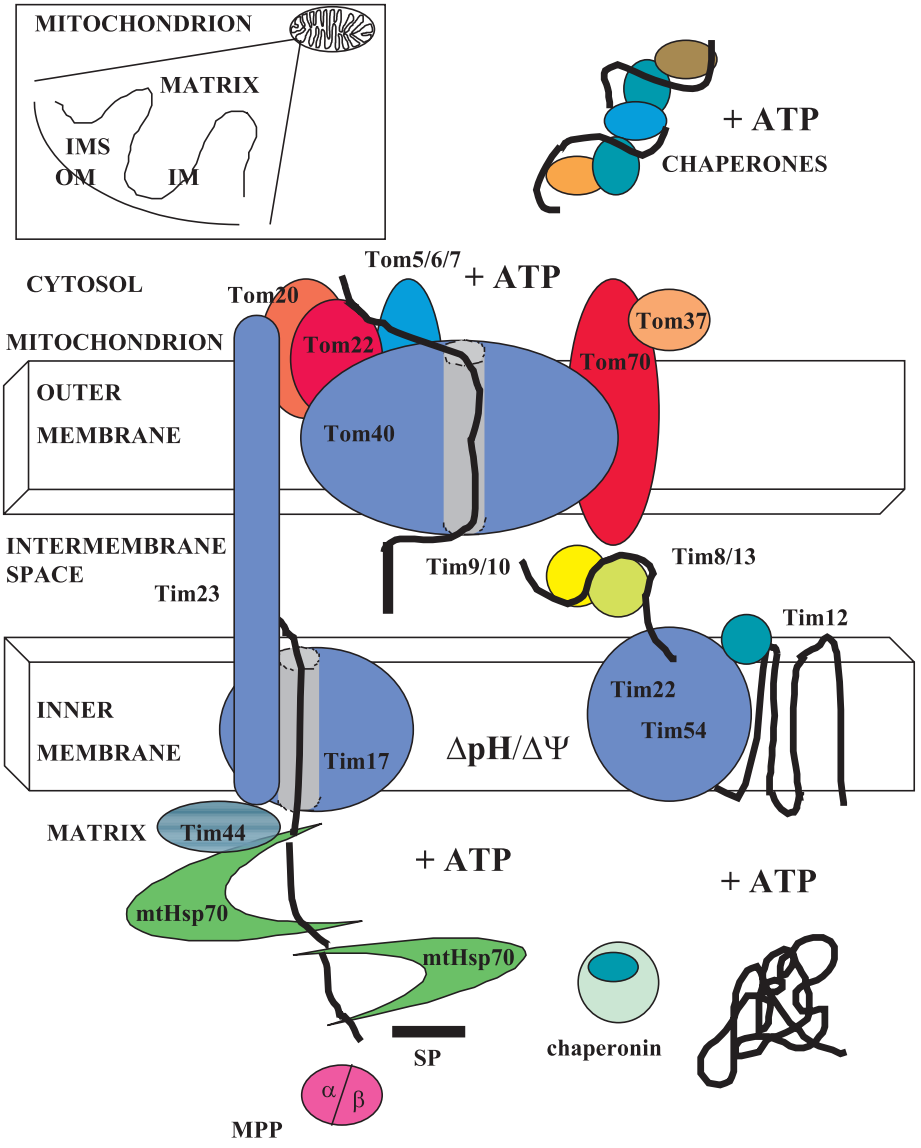

Fig. 6. Translocation of proteins into mitochondria. Mitochondria have four different compartments: the outer membrane (OM), inner membrane (IM), intermembrane space (IMS), and the matrix (upper left). Protein precursors are bound by a complex of molecular chaperones, and, in the presence of ATP, maintain the precursors in a transport-competent state. Tom20/22 and Tom37/70 bind to different substrate proteins. Tom5/6/7 modulate the translocation of substrate proteins across the translocon formed by Tom40. Proteins destined for the mitochondrial matrix are translocated across the inner mitochondrial membrane through a translocon formed by Tim23 and Tim17. Translocation requires a proton-motive force ($\Delta pH/\Delta\Psi$) across the inner membrane. Tim44 interacts with Tim23 and a mitochondrial heat shock protein of 70 kDa (mtHsp70) that hydrolyzes ATP and pulls the substrate protein into the mitochondrial matrix. The mitochondrial processing protease (MPP) cleaves the signal peptide (SP) from the substrate. Proteins that remain in the matrix will be refolded with the help of mtHsp70 and the chaperonin 60 kDa molecular chaperone. Proteins destined for residence in the inner membrane may first enter the matrix and then return to the inner membrane using a second signal sequence exposed after the first has been removed (not shown). Alternatively, inner membrane proteins may be directly targeted in a pathway requiring Tim9/10 and Tim8/13 in the intermembrane space. The inner membrane translocon used in this case is a complex of Tim22 and Tim54.

has yet to be determined. The sequential binding of the positively charged signal sequence with increasing affinity to acidic domains of the GIP proteins would allow the translocation of the signal sequence [131,132]. An acidic domain has also been identified in the intermembrane domain of the inner membrane protein Tim23 [133], the protein that actually spans both the inner and the outer membranes [109].

The signal sequence of matrix precursor proteins first interacts with the intermembrane space domain of the inner membrane protein Tim23, which together with Tim17 forms the translocation pore complex [134] (Fig. 6). Translocation across the inner membrane is dependent on the proton-motive force [135,136]. Bauer et al. [133] have shown that Tim23 can also regulate the opening of the pore. A third protein, Tim44, is also part of the inner membrane translocation process. Tim44 is a peripheral protein that binds to the intralumenal domain of Tim23 [137] (Fig. 6). Tim44 acts as the docking protein for the matrix chaperone mitochondrial Hsp70 (mtHsp70) and contains a DnaJ-like domain [138]. mtHsp70 is involved in both translocation and refolding of the translocated proteins [139]. These interactions are ATP-dependent [140,141]. The yeast mitochondrial nucleotide exchanger Mge1p interacts with the mtHsc70–preprotein complex during translocation across the inner membrane [142,143]. Disruption of the Tim44–mtHsp70 interaction has shown that binding of mtHsp70 to the preprotein is not enough to promote translocation of polypeptides that are not fully unfolded [144,145]. mtHsp70, when anchored to the membrane, may be capable of undergoing conformational changes induced by ATP binding and hydrolysis that result in the 'pulling' of the precursor protein into the matrix [146].

The removal of the signal sequence takes place in the matrix at an early import stage [147] (Fig. 6). The major signal protease is the matrix processing peptidase (MPP), a heterodimer formed by α- and β-subunits [148] (Fig. 6). Removal of the signal sequence is essential for the proper folding and function of matrix proteins [115].

Proteins destined to the inner mitochondrial membrane can first be targeted to the matrix and then imported into the inner membrane [76]. These proteins usually bind to the cytosolic face of Tom20, one of the receptors for matrix preproteins [122]. However, other inner membrane proteins bind to the surface receptor Tom70/Tom37 [123] (Fig. 6). The existence of secondary or alternative receptors for mitochondrial protein translocation explains the observation that deletion of neither Tom20 [149,150] nor Tom70 [151,152] is lethal. Similarly to mitochondrial matrix precursor proteins, the inner membrane preprotein interacts with Tom5, part of the GIP complex. During translocation across the outer membrane, the protein substrate interacts with both Tom40 and Tom22 which form the aqueous pore [153]. When the inner membrane protein emerges in the intermembrane space it associates with the intermembrane space complex formed by Tim9 and Tim10 [154]. The Tim9/10 complex seems to be responsible for driving the protein across the GIP into the intermembrane space [154,155]. In addition, two other soluble proteins in the intermembrane space, Tim13 and Tim8, have homology to Tim9 and Tim10 respectively [156], and they might associate with Tim9/10, but their function is not known [157]. Tim12 is a peripheral inner membrane protein facing the intermembrane space, and it is tightly associated with the Tim22/Tim54 complex [137,154] (Fig. 6). The mechanism of protein insertion into the inner membrane is not known, but it is dependent on a proton-motive force and the proteins Tim12, Tim22, and Tim54 [137,154,158].

Two interesting exceptions to the pathways described above are followed by the inner membrane proteins Tim22 and Tim54 that together participate in the insertion of inner membrane proteins. Tim22 binds to the surface receptor Tom20, instead of Tom70, and then is inserted through the Tim22/Tim54 complex. On the other hand, Tim54 binds to Tom70 and it is inserted into the inner membrane through the Tim23/Tim17 complex [159].

Outer membrane precursor proteins, such as porins, are also inserted through the GIP. Tom7 destabilizes the interactions between Tom40 and Tom22, and this effect allows for lateral movement and the insertion of the transmembrane protein into the lipid bilayer of the outer membrane [160]. Tom40 also uses the GIP complex for import into the outer membrane and integrates into preexisting Tom complexes [124]. Interestingly, Tom40 seems to enter the translocation pore partially folded as a requirement for proper insertion into the outer membrane [124].

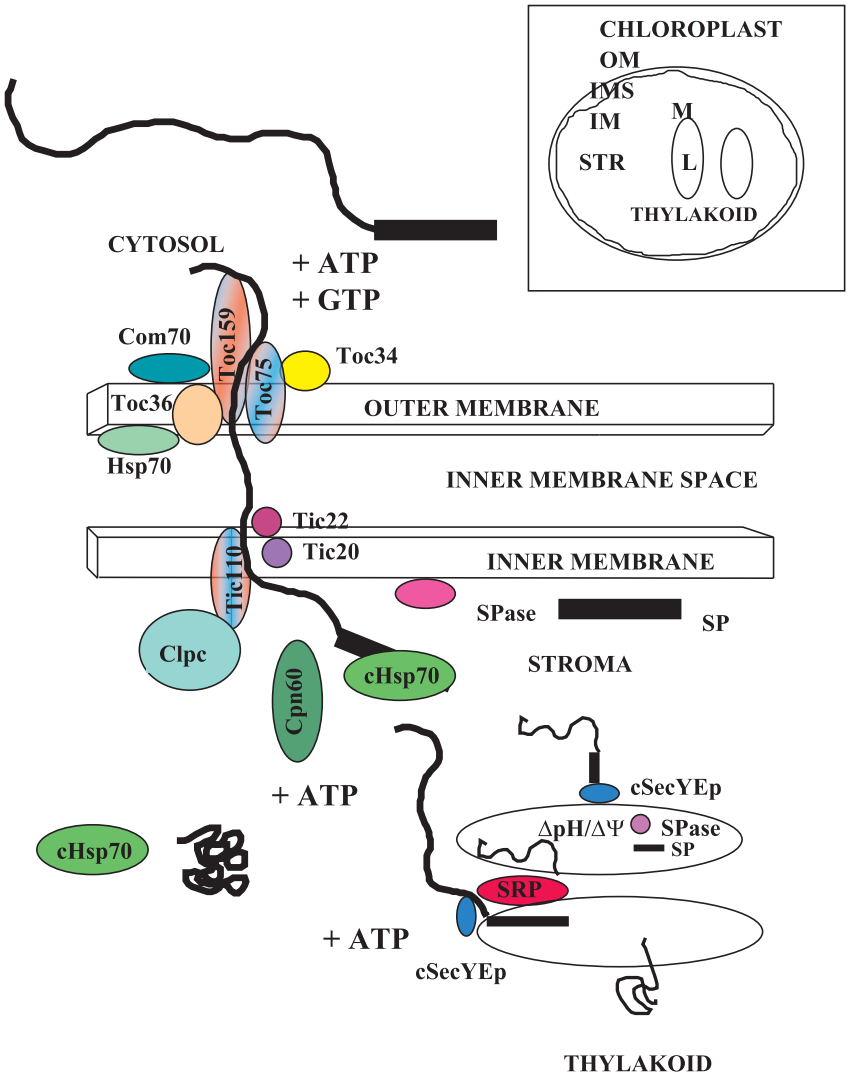

Fig. 7. Translocation of proteins into chloroplasts. Chloroplasts contain six different compartments: outer membrane (OM), inner membrane (IM), intermembrane space (IMS), stroma (STR), thylakoid membrane (M), and thylakoid lumen (L) (upper right). Substrate proteins contain signal sequences that are recognized by molecular chaperones tightly associated with the chloroplast outer membrane (Com70). The actions of Com70 as well as other aspects of the translocation process are stimulated by ATP (+ATP). Association of substrate proteins with the Toc159/75 translocon is stimulated in the presence of GTP (+GTP). An hsp70 at the inner surface of the outer membrane aids in the transfer of substrate proteins to the Tic complex. A chloroplast hsp70 in the stroma helps the precursor protein transit the membranes and also helps to refold proteins in the stroma. A signal peptidase (SPase) cleaves the signal peptide (SP). Other molecular chaperones including Clpc and Cpn60 may contribute to import of substrate proteins and to their folding in the stroma. Proteins can enter the thylakoid lumen or membrane by a variety of mechanisms related to the Sec pathway, the TAT pathway ($\Delta pH/\Delta\Psi$), the SRP pathway, or spontaneous insertion described for bacteria. cSecYEp, chloroplast homologues of SecYEp.

Protein residents of the intermembrane space, such as Tim8, Tim9, Tim10, and Tim13, are also transported through the GIP complex but they do not require the receptor proteins Tom22/Tom20. Instead, Tom5 is required for translocation of these proteins [159] (Fig. 6).

Finally, Oxa1p, a homologue of bacterial YidC, exists in mitochondria and is required for the insertion of certain inner membrane proteins after their import and processing within the mitochondrial matrix [161].

3.3. Chloroplast protein translocation pathways

Chloroplasts have at least six distinct compartments: outer membrane, intermembrane space, inner membrane, stroma, thylakoid membrane, and thylakoid lumen (Fig. 7). Each compartment contains specific proteins. For example, the thylakoid membranes within the chloroplast contain the proteins responsible for photosynthesis and electron transport. Chloroplasts, like mitochondria, contain their own genome, but most chloroplast proteins are synthesized on cytosolic ribosomes and are imported post-translationally. These proteins are synthesized with targeting sequences that are subsequently cleaved from the proteins after import. These targeting sequences are highly variable in length (from 20 to more than 120 amino acids) but contain basic amino acids and a high content of serine and threonine [162,163]. These targeting sequences do not fold into secondary or tertiary structures in an aqueous environment, but form amphipathic β-strands or α-helices in a hydrophobic environment [164,165].

Protein translocation across the outer and inner membrane occurs simultaneously for most proteins, probably at regions where the outer and inner membranes are in close contact [162,164]. The translocon at the outer membrane of chloroplasts (Toc) binds to a substrate protein and transfers the protein to the translocon at the inner chloroplast membrane (Tic). This process is greatly stimulated by ATP (Fig. 7). Without ATP, only weak binding of substrate proteins to Toc159 and Toc75 occurs [162]. In addition to being receptors, Toc75 and Toc159 form the aqueous pore through which the precursor protein translocates [164]. The diameter of this pore is only 8–9 Å suggesting that proteins must be fully unfolded to translocate into chloroplasts [162]. Toc159 and Toc34 also bind GTP and have intrinsic GTPase activities (Fig. 7). Toc36 is required for optimal rates of protein translocation, but its mechanisms of action are not yet known. The chloroplast outer membrane protein of 70 kDa (Com70) is an Hsp70 family member that is tightly associated with the outer membrane [166] (Fig. 7). It binds to substrate proteins at an early stage of translocation and is an especially active protein unfoldase in the presence of ATP (Fig. 7). Another Hsp70 family member is associated with the inner face of the outer membrane and plays a role in delivery of substrate proteins from the Toc complex to the Tic complex [162,164] (Fig. 7).

Tic is composed of Tic110, Tic22, and Tic20 (Fig. 7). Tic110 can bind to precursor proteins, and the proteins translocate across the inner membrane in close association with Tic20 and Tic22 [167]. Tic110, Tic20, and Tic22 do not form a stable association except when interacting with the Toc complex. Insertion of the precursor protein into the Tic complex requires ATP hydrolysis within the stroma [162] (Fig. 7). Interestingly, Tic22 enters the intermembrane space of the chloroplast by a mechanism completely different than that described for stromal proteins [168]. Tic22 does not compete for chloroplast translocation with any other precursor protein examined. Whether or not other intermembrane space chloroplast proteins follow this unique translocation pathway remains to be established [168].

When the precursor protein reaches the stroma, a signal peptidase removes the precursor sequence [162]. The molecular chaperones Clpc and Cpn60 associate with Tic110. Clpc and the chloroplast Hsp70 (cHsp70) may be required for protein transport across the inner membrane, while Cpn60 assists in protein refolding in the stroma [162–164].

Many proteins including ferredoxin and ribulose 1,5-bisphosphate carboxylase reside in the chloroplast stroma after import, but others enter the thylakoid membrane or lumen. Mechanisms of protein translocation into the thylakoid membrane of chloroplasts are numerous and resemble the various protein export pathways in bacteria [68,169]. Some proteins including the light harvesting chlorophyll-binding protein enter the thylakoid lumen using components homologous to the Sec apparatus. These proteins often have a second targeting sequence that is exposed at the amino terminus after the initial precursor has been cleaved by the stromal signal peptidase (Fig. 7). Proteins containing very hydrophobic thylakoid targeting sequences also require a stromal homologue of SRP. The import of both of these signal containing proteins is by SecY [170] and SecE [171] homologues in the thylakoid membrane and a SecA homologue [172,173] in the chloroplast stroma. The translocation requires ATP hydrolysis (Fig. 7). Other proteins are imported into the thylakoid lumen by a pathway similar to the TAT path-

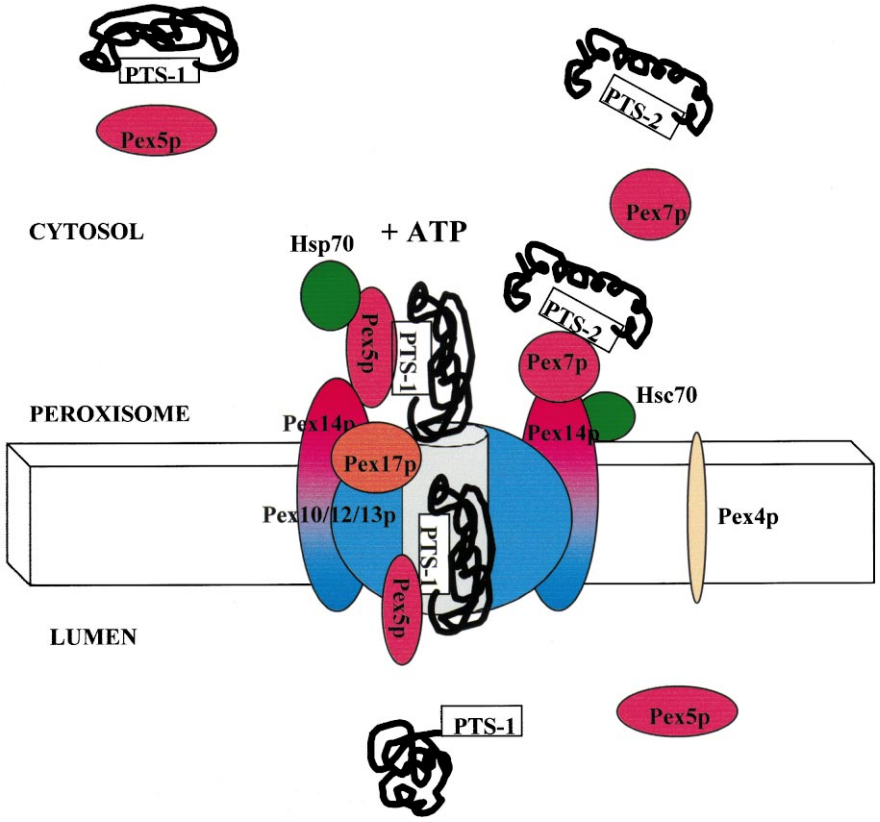

Fig. 8. Translocation of proteins into peroxisomes. Substrate proteins contain PTS-1 or PTS-2 targeting sequences. Different cytosolic receptors, Pex5p and Pex7p, recognize these sequences and direct the proteins to peroxisome receptors, Pex17/14p or Pex14p and translocons, Pex10/12/13p. Hsp70s and ATP (+ATP) stimulate translocation. The PTS-1 receptor, Pex5p, enters the peroxisome along with substrate proteins, and proteins cross the membrane in a folded state. The Pex5p is recycled to the cytosol. No role for molecular chaperones in the lumen of the peroxisome has yet been proved.

way for export of bacterial proteins. These proteins are transported in a folded state, and they contain a twin arginine motif in their targeting sequences [169]. The pH of the chloroplast stroma is 8.0 while that of the thylakoid lumen is 5.0, and the TAT translocation process is dependent upon the proton-motive force. There is also evidence for a ΔpH-dependent translocon in the thylakoid membrane [65]. Finally, several proteins involved in photosynthesis and electron transport insert into the thylakoid membrane by mechanisms that are independent of the Sec, SRP, or the TAT pathways (Fig. 7). Some of these proteins may require Albino3, a homologue of bacterial YidC, localized within chloroplasts [174].

3.4. Peroxisomal protein import pathways

Peroxisomes are surrounded by a single membrane, and some of the proteins within that membrane may be targeted to peroxisomes through the ER and Golgi by means of vesicular traffic [175]. However, most peroxisomal membrane proteins are synthesized in the cytosol and inserted into the membrane posttranslationally [176]. This latter pathway requires initial binding of the protein to another peroxisomal membrane protein prior to insertion into the lipid bilayer [176]. The insertion is temperature-dependent and requires ATP for certain peroxisomal membrane proteins but not others [176]. The mechanisms of import of peroxisomal membrane proteins remain an important gap in our understanding of protein translocation.

Peroxisomal matrix precursor proteins are synthesized in the cytosol and then imported into peroxisomes [177]. Peroxisomal proteins are designated by a unified nomenclature as 'peroxins', and the genes

involved in peroxisomal biogenesis are represented by the acronym, PEX [178]. Proteins can be translocated into the peroxisomal matrix in a folded state [179,180], a feature shared with the bacterial TAT translocation pathway and its homologous pathway for translocation of proteins into or across the thylakoid membrane of chloroplasts.

Genetic and biochemical studies indicate that two classes of signal sequences are responsible for targeting of peroxisomal matrix precursors (Fig. 8). The peroxisome targeting signal 1 (PTS-1) is the most common signal peptide in proteins destined to the peroxisome lumen [64]. PTS-1 is a tripeptide present at the carboxyl terminus of the polypeptide, and it is loosely based on the sequence serine-lysine-leucine (SKL) [181]. Further studies have shown that alanine or cysteine can substitute for serine, arginine and histidine can substitute for lysine, and methionine can replace leucine, so the PTS-1 is more accurately described as S/A/C-K/R/H-L/M [182]. The PTS-1 targeting peptide is not removed from the protein after import into the peroxisome lumen (Fig. 8).

The peroxisomal targeting sequence 2 (PTS-2) is found near the amino terminus of polypeptides such as 3-keto-acyl-coenzyme A thiolase and consists of a peptide of nine amino acids with the consensus sequence arginine/lysine-leucine/isoleucine/valine-x_5-histidine/glutamine-leucine/alanine (R/K-L/I/V-X-X-X-X-X-H/Q-L/A) [64,176]. PTS-2 targeting sequences are active when engineered to be at locations within the protein sequence other than the amino terminus. PTS-2 targeting signal sequences are removed after entry into the peroxisome by a signal peptidase in plants and mammals but not in yeast [176]. Targeting of integral peroxisomal membrane proteins mentioned earlier is independent of both PTS-1 and PTS-2 pathways [176,183,184].

The peroxisomal protein Pex5p has been identified as the receptor for the PTS-1 signal sequence [178,185–187] (Fig. 8). Upon binding of the receptor to the substrate protein, the receptor–substrate protein complex is targeted from the cytosol to the peroxisomal membrane where Pex5p binds to the integral peroxisomal membrane protein Pex13p [188,189] and/or the membrane protein Pex14p [190–192] (Fig. 8). The proteins that actually form the translocon in peroxisomal membranes have not been conclusively identified. However, Pex13p in the peroxisomal membrane appears to be in a complex with Pex12p and Pex10p, and deletions of Pex12p or Pex10p block protein import at steps subsequent to substrate binding [181,184,193,194]. The peripheral membrane protein Pex17p binds to Pex14 [192] and has also been implicated in the Pex5p receptor binding [188–190,192]. Therefore, we speculate that the peroxisome translocon may be formed by Pex10/12/13/17 (Fig. 8).

Pex5p actually enters the peroxisomal lumen with PTS-1 substrate proteins (Fig. 8). However, the frequency with which Pex5p enters the peroxisome is not known. Interestingly, Pex4p is a peroxisomal membrane protein that may participate in the recycling of the Pex5p receptor back into the cytosol since the ΔPEX4 deletion results in Pex5p accumulating within the peroxisome lumen [195]. This entry of the receptor along with the substrate protein into the organelle may also apply to the lysosomal uptake of proteins (see below).

Pex7p, the PTS-2 receptor, is mostly localized to the peroxisomal membrane and specifically binds to PTS-2 signal sequences and targets the precursors to the peroxisome membrane [178,196–198]. Pex7p is able to bind to the peripheral membrane protein Pex14p [192] which interacts with the integral membrane protein Pex13p [176]. Therefore, Pex14p seems to be the site of convergence of both PTS-1 and PTS-2 pathways [190]. Both PTS-1 and PTS-2 targeted peroxisomal proteins share the core translocon consisting of Pex10/12/13p (Fig. 8). It is not yet known whether or not Pex7p, like Pex5p, enters the peroxisome along with substrate proteins.

Both Hsp70 and the heat shock cognate protein of 70 kDa (Hsc70) are associated with the cytosolic side of the membrane of purified rat liver peroxisomes [199] (Fig. 8). Microinjection of anti-Hsc70 antibodies into intact cells, or depletion of Hsc70 from cytosol added in a permeabilized cell assay significantly inhibited peroxisomal transport [199,200]. Members of the Hsp70 family have also been observed in the lumen of plant peroxisomes (glyoxisomes) [201,202]. In addition, a plant Hsp70 interacts with a peroxisomal membrane-anchored DnaJ/Hsp40 homologue [202]. Further fractionation of purified peroxisomes from cucumber cotyledons indicates that two isoforms of Hsp70 and a soluble form of the Dna-J/Hsp40 homologue were present in the lumen of per-

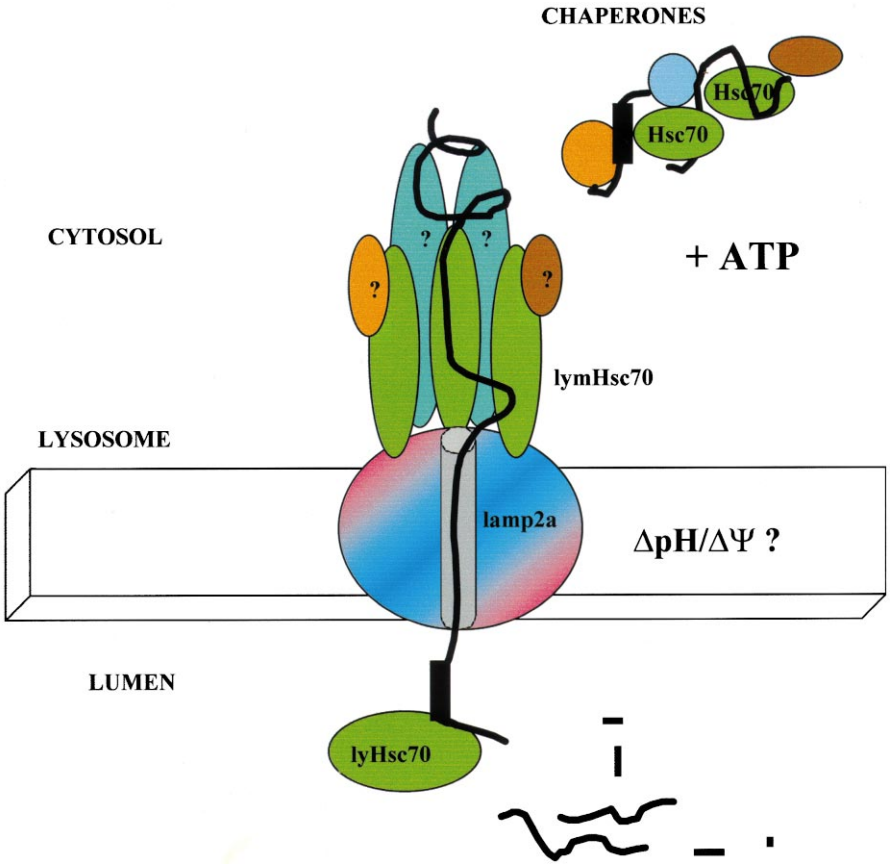

Fig. 9. Protein translocation into lysosomes. Substrate proteins contain a targeting peptide that is recognized in an ATP-dependent manner by a molecular chaperone complex including Hsc70. This complex of chaperones is also associated with the lamp2a in the lysosomal membrane. An hsp70 in the lysosomal lumen (lyHsc70) is required to pull the substrates into the lysosome, but a requirement for ATP has not yet been shown. A requirement for a proton-motive force in protein translocation is unknown ($\Delta pH/\Delta\Psi$?). Soon after import the protein is degraded by the high concentrations of cathepsins in the lysosome.

oxisomes [202]. On the other hand, no Hsp70 protein was found in the peroxisomal lumen from rat liver peroxisomes [199]. These contradictory results could be explained by actual differences between the mammalian and the plant systems or by differences in the sensitivity of the techniques and reagents used for the immunodetection. A requirement for molecular chaperones in the targeting of matrix proteins to peroxisomes is somewhat surprising since protein unfolding is not required for this protein translocation pathway [179,180]. Perhaps the requirement for molecular chaperones is for their role in facilitating assembly of protein complexes.

3.5. Protein translocation into lysosomes

Lysosomes are able to take up and degrade proteins by several pathways involving vesicular traffic [203]. Exogenous proteins as well as membrane proteins can be delivered to lysosomes by endocytosis [1,2]. Secretory proteins can be delivered to lysosomes for degradation when the secretory vesicle fuses with a lysosomal membrane instead of the plasma membrane. This process, crinophagy, is often activated when the demand for the secreted protein is low [204]. For example, crinophagy of insulin by the insulin producing β-cells increases when blood glucose levels are low. Under these circumstances demand for insulin secretion is low. Cytosolic and organelle proteins can be taken up by lysosomes by the processes of macroautophagy and microautophagy. In macroautophagy, regions of cytoplasm are first surrounded by a double membrane to form an autophagosome, and many of the mechanisms responsi-

ble for this process have been recently discovered [6,7]. The autophagosome acidifies and then fuses with lysosomes. Microautophagy refers to the indentation of the lysosomal membrane to form tubules which then pinch off to yield vesicles within the lysosome [205,206]. The membrane of such vesicles breaks down to release internalized materials into the lysosomal lumen.

In addition to these vesicular pathways, lysosomes are able to take up cytosolic proteins for degradation in a molecule-by-molecule fashion [203,207]. This process, chaperone-mediated autophagy, is activated by prolonged starvation or by the removal of serum growth factors from confluent cells in culture [208–210]. This pathway of proteolysis is similar to the other protein translocation systems discussed in this review. Substrate proteins contain targeting sequences related to lysine-phenylalanine-glutamate-arginine-glutamine (KFERQ) [211–214], and Hsc70 and cochaperones stimulate this proteolytic pathway [215–218] (Fig. 9). At least one role of the molecular chaperone is to unfold protein substrates [219]. Dihydrofolate reductase (DHFR) is a substrate for chaperone-mediated autophagy, and methotrexate is known to stabilize the conformation of DHFR. Methotrexate markedly inhibits transport of DHFR into lysosomes but does not affect its binding to the lysosome surface [219]. These results suggest that proteins must be unfolded to translocate across the lysosomal membrane.

Molecular chaperones within the lysosomal lumen are required for the import of substrate proteins [220,221] (Fig. 9). Most substrate proteins bind to a receptor at the lysosomal surface, the lysosome-associated membrane protein 2a (lamp2a) [222]. Lamp2a levels in the lysosomal membrane are dynamically regulated [208], and the level of lamp2a directly correlates with the activity of the pathway under a wide variety of physiological and pathological conditions [223].

The translocon in the lysosomal membrane has not yet been conclusively identified, but it may be that the lamp2a receptor also forms the protein translocation channel through the membrane. Lamp2a multimerizes into tetramers, octamers, and larger homomultimers [223] and so could provide multiple membrane spanning protein segments.

We have identified two variants of Hsc70 that dif-

Table 1
Properties of the different protein translocation systems in this review

Translocation system	ATP/GTP	$\Delta pH/\Delta\Psi$	Mol. chaps.	Substrate unfolding	SPase
Prokaryotic					
Inner membrane, Sec	ATP	yes	yes[a]	yes	yes
Inner membrane, SRP	GTP	yes	no	yes[b]	yes
Inner membrane,TAT	no	yes	?	no	yes
Eukaryotic					
ER, SRP	ATP+GTP	no	yes[b]	yes[b]	yes
ER, posttranslational	ATP	no	yes	yes	yes
Mitochondria, outer membrane	ATP	no	yes	yes	no
Mitochondria, inner membrane	ATP	yes	yes	yes	yes
Chloroplast, outer membrane	ATP+GTP	no	yes	yes[c]	no
Chloroplast, inner membrane	ATP	no	yes	yes[c]	yes
Chloroplast, thylakoid membrane	ATP+GTP[d]	yes	yes	yes	yes
Peroxisome membrane	ATP	no	yes	no	no[e]
Lysosome membrane	ATP	?	yes	yes	no

$\Delta pH/\Delta\Psi$ refers to a requirement for a proton-motive force across the membrane. SPase refers to whether or not translocated proteins are cleaved by a signal peptidase.

[a]SecAp and SecBp are both receptors and molecular chaperones.

[b]The substrate is unfolded as a growing polypeptide chain.

[c]Com70 is a particularly strong unfoldase.

[d]ATP and GTP are required for Sec- and SRP-dependent protein translocation into the thylakoid membrane, but not for translocation by the TAT pathway.

[e]A few PTS-2 containing proteins are cleaved in mammals and plants, but not in yeast.

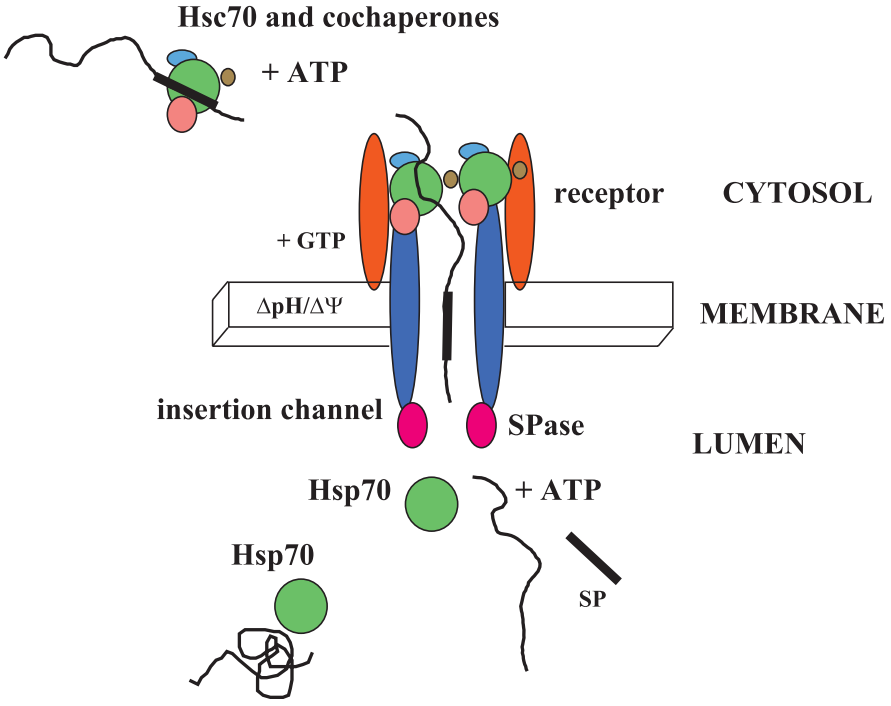

Fig. 10. Composite pathway for protein translocation across membranes. Protein translocation commonly requires a targeting peptide motif in substrate proteins, a molecular chaperone complex in the cytosol, and ATP hydrolysis. The cytosolic molecular chaperones often bind to organelle membrane proteins as well as to substrate proteins. GTP and proton-motive force ($\Delta pH/\Delta\Psi$) are required in some translocation systems but not others. Receptors for substrate proteins dock with integral membrane proteins forming the protein insertion channel or translocon. Molecular chaperones within the organelle (Hsp70) pull the substrate protein across the organelle membrane. A signal peptidase (SPase) cleaves the signal peptide (SP) after the protein enters the organelle lumen. In some cases the organelle molecular chaperone assists the protein in refolding.

fer in their p*I* and location in the lysosome [224]. The most acidic variant (p*I* = 5.3) is localized within the lysosomal matrix (lyHsc70) while the less acidic form (p*I* = 5.5) is associated with the cytosolic face of the lysosomal membrane (lymHsc70). Both variants of Hsc70 are required for transport of substrate proteins [220]. We are characterizing several other polypeptides that form a large molecular weight complex with lymHsc70 (Fig. 9).

This complex could participate in maintaining a tight seal of the translocation complex in a similar manner as described for the translating ribosome bound to the Sec61p complex in the ER on the cytosolic side of the membrane [225]. At the same time it might act as a receptor for certain substrates and that might not require additional stable binding to lamp2a. Finally, this complex at the lysosomal membrane is likely to be responsible for the unfolding of protein substrates known to be required for their transport into lysosomes by this pathway [219].

Cuervo and Dice have recently determined that a fraction of lamp2a is in the lumen of the lysosome forming a lipid–protein complex perhaps with cholesterol [208]. They proposed a dynamic model where the lumenal lamp2a can be recruited to the lysosomal membrane upon activation of chaperone-mediated autophagy. It is possible that cholesterol micelles containing lamp2a intercalate into the lysosomal membrane and then lamp2a incorporates into preexisting translocation complexes by lateral diffusion. This model would explain the fact that upon activation of chaperone-mediated autophagy the levels of lamp2a increase [217,223]. The concentration dependence of multimerization of a transmembrane protein may not be linear so that the amount of octamers may increase dramatically in response to a 2-fold increase in lamp2a in the lysosomal membrane.

The interaction of protein substrates with lamp2a most likely positions these substrates for insertion

Table 2
Receptor and translocon components of membrane translocation systems

Translocation system	Receptors	Translocons
Prokaryotic		
Inner membrane, Sec	SecBp, SecAp	SecYp,Ep,Ap
Inner membrane, SRP	SRP, FtsY	SecYp,Ep
Inner membrane, TAT	TAT A, E, B, D	TAT C
Eukaryotic		
ER, SRP	SRP, SR	Sec61α,β,γp, TRAM
ER, posttranslational	Sec71/72p, Sec62/63p	Sec61α,β,γp
Mitochondria, outer membrane	Tom70/37, Tom20/22	Tom40
Mitochondria, inner membrane	Tim9/10, Tim8/13	Tim54/22, Tim23/17
Chloroplast, outer membrane	Toc159, Toc75	Toc159, Toc75
Chloroplast, inner membrane	Tic110	Tic110, Tic20/22
Chloroplast, thylakoid membrane	TATs	TATs
Peroxisome membrane	Pex5p, Pex7p, Pex14p	Pex10/12/13p, ?
Lysosome membrane	lamp2a	lamp2a

The translocons listed are the minimal components required for proteins to be able to translocate across membranes.

into the translocation pore. A similar interaction has been proposed between Tom5 and preproteins on the outer membrane of the mitochondrion [112] and for Toc159 on the outer membrane of chloroplasts [162].

3.6. Common themes in protein translocation across membranes

The different protein translocation systems reviewed have several common themes that are summarized in Table 1. Many translocation pathways require ATP and/or GTP. A notable exception to this requirement is the TAT pathway in bacteria and the evolutionarily conserved pathway into or across the chloroplast thylakoid membrane. A requirement for a proton-motive force is also common but not universal. Alternative driving forces must exist in many translocation systems. A role for molecular chaperones in the cytosol, in the organelle lumen, and even in the organelle membrane is a common feature even when protein substrate unfolding is not required such as in peroxisomal protein translocation. In such cases additional roles for molecular chaperones such as in targeting substrate proteins or regulating import machinery need to be considered. The presence of peptide signals in substrate proteins and the removal of these signal sequences after import is also a common, but not universal, finding. Such targeting peptides exist for chaperone-mediated autophagy, for example, but removal of such sequences is not necessary in a protein degradation pathway in which substrate proteins do not have to function within the organelle.

We have also tabulated the receptors and translocon components for the various protein translocation systems (Table 2). Usually the receptors and translocons are composed of distinct proteins. However, in the chloroplast inner membrane the Tic110 receptor also participates in forming the translocon, and in the outer membrane the receptor and translocon proteins are the same. These considerations make it somewhat less unusual that lamp2a in lysosomes may act as both receptor and translocon (Table 2).

The size of the opening in the translocon that would be needed to allow an unfolded protein to translocate is difficult to predict based on the other protein translocation systems reviewed. The size of the pore of the ER translocon ranges from approx. 15 Å when closed, to 40–60 Å when translocation is initiated [105]. Such changes in diameter of the central pore of the translocon might indicate a mechanism of expansion and contraction of the translocation complex [29]. A similar 'iris' mechanism was recently proposed for the TAT transporter, where proteins are arranged in concentric circles defining a ring-like structure, and the diameter of the pore is modified by the individual polypeptides sliding against each other [75]. Based on mass determination

by density gradient centrifugation of purified complexes, and measurements from electron micrographs, the ER translocon appears to be composed of three to four Sec61 trimers [226]. Therefore, the ER translocon would be defined by over 40 transmembrane domains [89]. Similar conclusions were reached by Rapoport and colleagues [29] concerning the *Bacillus subtilis* translocon.

On the other hand, the translocon opening size across the chloroplast outer membrane was estimated to be only 8–9 Å in diameter even in the active state of protein translocation [162], and the GIP of the mitochondrial outer membrane forms a pore of 20 Å diameter [76,121,227]. Clearly, protein translocation can be accomplished using protein translocons with widely varying opening capacities.

Based on these observations we could calculate the minimum number of transmembrane domains supplied by multimerized lamp2a required to form a possible translocation pore by dividing the perimeter of the pore by the diameter of each transmembrane domain. Taking into account that the average diameter of a transmembrane domain is 12 Å [89], a minimum of eight transmembrane domains is required to form a pore of 15 Å in diameter, and six could form a pore of 9 Å in diameter.

The common features of the protein translocation systems can be blended into a generic mechanism for translocating proteins across membranes (Fig. 10). Such a system for general protein translocation would require chaperone complexes and ATP hydrolysis in the cytosol and in the organelle lumen. The minimal translocon consists of receptor and insertion channel proteins, perhaps even as separate domains of a single protein, and a signal peptidase that cleaves the targeting sequence as it enters the organelle. Requirements for GTP and a proton-motive force are also possible. Perhaps such a prototype protein translocation system has been embellished in eukaryotic organelles to add specificity to avoid mistargeting of proteins.

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