Common Principles of Protein Translocation Across Membranes

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Common Principles of Protein Translocation Across Membranes

Gottfried Schatz* and Bernhard Dobberstein

Most major systems that transport proteins across a membrane share the following features: an amino-terminal transient signal sequence on the transported protein, a targeting system on the cis side of the membrane, a hetero-oligomeric transmembrane channel that is gated both across and within the plane of the membrane, a peripherally attached protein translocation motor that is powered by the hydrolysis of nucleoside triphosphate, and a protein folding system on the trans side of the membrane. These transport systems are divided into two families: export systems that export proteins out of the cytosol, and import systems that transport proteins into cytosol-like compartments.

A protein's function depends critically on its correct subcellular location. Cells have therefore developed elaborate systems for maintaining membrane-limited compartments endowed with specific proteins. Roughly one-third of a cell's proteins are membrane proteins, even in *Mycoplasma genitalium* that must get by with only 482 genes (*1*). Also, many soluble proteins must travel across one or two membranes to reach their final location, either outside the cell or within an intracellular compartment (*2*). Thus, almost half of the proteins of an average cell are transported into or across a membrane.

Export and Import Systems

There are two major types of protein transport systems (Fig. 1). One type is located in the bacterial plasma membrane, the endoplasmic reticulum, and the internal membranes of mitochondria and chloroplasts. We refer to it as an export system because it exports proteins from the cytosol into extracytosolic compartments (the bacterial periplasm, the lumen of the endoplasmic reticulum, the thylakoid lumen, or the mitochondrial inner membrane). The other type of system is located in the outer and inner membranes of mitochondria and chloroplasts and in the peroxisomal membrane. We refer to it as an import system because it transports proteins into compartments that are functionally equivalent to, or evolutionarily derived from, the cytosol (the inner compartments of mitochondria, chloroplasts, and peroxisomes) (*2*, *3*). Mitochondria and chloroplasts thus have export as well as import systems.

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The export and import systems are general systems that can transport many different proteins. Membranes may also contain special systems that transport only a few proteins, but these systems are quite different from the general systems and shall not be considered here. We shall also not consider protein transport through the nuclear pores because the transported proteins never leave the cell's cytosolic phase [see the related article by Görlich and Mattaj (*4*) in this issue]. Finally, we shall treat the protein transport system of peroxisomes separately, as it appears to work quite differently from the other systems.

Export and import systems operate by a similar set of principles (*2*, *5*): The transported protein usually carries an NH_2-terminal signal and is recognized by cytosolic factors that deliver it to specific receptors on the target membrane; the protein usually remains partly unfolded during transport; the signal sequence opens a hetero-oligomeric transmembrane channel that is gated both across and within the plane of the membrane; the channel is coupled to a peripherally attached protein translocation motor that is driven by the hydrolysis of nucleoside triphosphate and transports the protein across the membrane; and the protein folds on the trans side of the membrane with the help of chaperones or folding enzymes. In discussing these different points, we will focus on general principles rather than on details in order to present a unified view of how proteins are translocated across membranes.

Targeting Signals

Proteins destined to be transported into or across a membrane are usually synthesized with an NH_2-terminal sequence that is proteolytically removed on the trans side of the membrane (*6*). These NH_2-terminal sequences have been termed signal-, leader-, targeting-, transit-, or presequences (*2*); we will refer to them as signal sequences. In general, export signal sequences are hydrophobic and import signal sequences more hydrophilic. Export signal sequences can

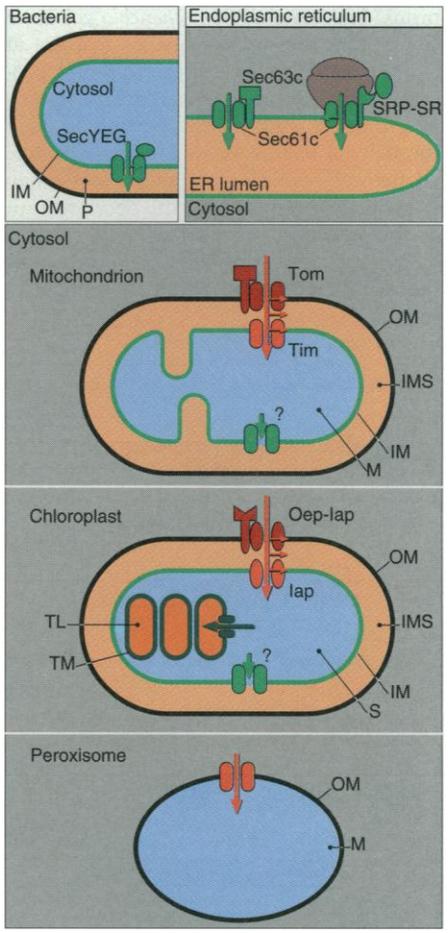

Fig. 1. Protein translocation systems. Export systems (green) export proteins from a cytosol to an extracytosolic compartment. Import systems (red-brown) import proteins into a space that is functionally equivalent to, or evolutionarily derived from, a cytosol. The import systems of mitochondria and chloroplasts consist of coupled systems in the outer (OM) and the inner membrane (IM). Reversible dissociation of the two systems allows protein import into the intermembrane space (IMS). In chloroplasts, some imported as well as organelle-encoded proteins are inserted into thylakoids, and perhaps also into the inner membrane, by an export system. In mitochondria, virtually all organelle-encoded proteins are inserted into the inner membrane by an unknown system, most likely an export system. The import system in the single membrane of peroxisomes is poorly understood. P, periplasm; M, matrix; S, stroma; TL, thylakoid lumen; TM, thylakoid membrane; ER, endoplasmic reticulum; Sec63c, Sec63 complex; Sec61c, Sec61 complex; SRP, signal recognition particle; SR, SRP receptor; SecYEG, Tim, Tom, Oep, and Iap, protein subunits of the translocation systems.

usually be interchanged within a given export system and can often be interchanged between different export systems. In contrast, import signal sequences can usually only be interchanged within the same organellar system (Fig. 2).

Signal sequences increase the specific interaction of a protein with its appropriate transport machinery by different mechanisms. Experiments in *Escherichia coli* have shown that the signal sequence may act at two distinct steps within the transport process. In the first step, it may retard folding of a protein, causing the protein to bind through its mature region to the cytosolic chaperone SecB (see below). SecB then specifically interacts with the SecA subunit of the transport machinery in the plasma membrane, delivering the bound protein to the membrane-linked SecYEG complex (a complex of SecY, SecE, and SecG subunits). In the second step, the signal sequence interacts with and activates the membrane-associated export machinery. In this mechanism the signal sequence is not a targeting signal but a folding inhibitor and a tag by which the membrane-linked transport machinery identifies a protein destined for transport (*7*, *8*). If the signal sequence is removed, export is inhibited 100- to 1000-fold, but export can be increased to almost normal rates by mutations in the SecYEG subunits (*9*). In other cases, however, the signal sequence acts as a true targeting signal because it is specifically recognized by a cytosolic chaperone or targeting factor (*10*).

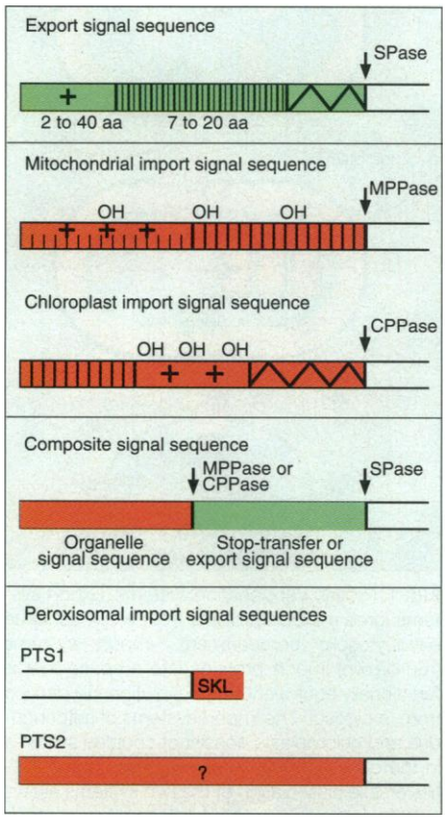

Fig. 2. Signal sequences. Export signal sequences are in green, import signal sequences in red. Abbreviations: aa, amino acid; SPase, signal peptidase; MPPase, mitochondrial processing peptidase; CPPase, chloroplast processing peptidase; PTS1 and PTS2, COOH- and NH_2-terminal import signal sequences for peroxisomes; OH, hydroxylated amino acids; vertical lines, hydrophobic region; zigzag lines, β strand; +, positively charged amino acid; SKL, Ser-Lys-Leu. The short vertical bars within the NH_2-terminal part of mitochondrial signal sequence indicate the hydrophobic face of an amphiphilic helix. The drawing of the composite signal sequence does not indicate the special features of each substructure.

Export signal sequences mediate the transport of protein across the bacterial plasma membrane, into the endoplasmic reticulum, into thylakoids, and into the mitochondrial inner membrane. They may also determine whether transport into the endoplasmic reticulum occurs co- or post-translationally, or whether transport into thylakoids is driven by adenosine triphosphate (ATP) or by a pH gradient (*10*, *11*) (see also below). Finally, if an export signal sequence is not cleaved off, and if its hydrophobic core is about 20 residues long, it can function as a signal-anchor sequence that permanently anchors the protein within the membrane, generating a transmembrane protein.

A transmembrane protein can also be generated by the sequential function of a signal sequence and a downstream hydrophobic stop-transfer sequence composed of about 20 amino acids. The signal sequence initiates translocation, and the stop-transfer sequence then probably triggers a lateral opening of the translocation channel. By escaping from the translocation channel, the stop-transfer sequence arrests translocation and becomes the transmembrane anchor of an integral membrane protein (*2*).

Import signal sequences transport proteins into the mitochondrial matrix or the chloroplast stroma. They are usually between 20 and 35 residues long, more hydrophilic than export signal sequences, and rich in hydroxylated amino acids. Mitochondrial import signal sequences are, in addition, rich in basic amino acids, generally lack acidic ones, and can fold into an amphiphilic α helix or α sheet (*6*). The basic and amphiphilic character of mitochondrial import signal sequences is essential for their function. The import signal sequences of chloroplasts are neither strongly basic nor amphiphilic, and their distinguishing features are still somewhat mysterious. Some import signal sequences transport a protein both into mitochondria and chloroplasts (*12*). Such a dual targeting shows that import signal sequences of mitochondria and chloroplasts are functionally related.

There also exist composite signal sequences in which two signal sequences act in tandem. For example, the import of proteins from the cytoplasm into thylakoids, the mitochondrial intermembrane space, or the mitochondrial outer membrane is often effected by an NH_2-terminal import signal sequence followed by an export signal sequence or a stop-transfer sequence. In chloroplasts, the import signal sequence directs the protein to the stroma where it is cleaved off; the export signal sequence then transports the protein into thylakoids. In mitochondria, the import signal sequence directs the protein to mitochondria, and a stop-transfer sequence then arrests translocation either in the outer or the inner membrane. A stop-transfer sequence in the mitochondrial outer membrane generates a protein spanning the outer membrane, and in the mitochondrial inner membrane stop-transfer generates a protein spanning the inner membrane, and proteolytic cleavage of this inner membrane protein may then generate a soluble protein of the intermembrane space (*13*). Export signal sequences and stop-transfer sequences acting within chloroplasts and mitochondria are less hydrophobic than the corresponding sequences of bacteria or the endoplasmic reticulum, perhaps because the organellar sequences must be transported completely across the organellar outer membrane.

Signal sequences specific for a given membrane transport system lack a strict consensus sequence. In fact, up to 25% of randomly generated peptides can function as signal sequences for the endoplasmic reticulum, mitochondria, and the bacterial plasma membrane (*14*). Thus, it is generally assumed that signal sequences have highly degenerate primary sequences and that their group-specific properties reflect a common secondary structure or a similar distribution of charged and apolar residues. However, the sequence degeneracy of naturally occurring signal sequences may be overestimated. Most authentic signal sequences lead to almost quantitative translocation of the attached protein across the target membrane, whereas most randomly generated sequences are much less effective. Also, a signal sequence mediates not only recognition of a protein by the membrane-linked transport system, but it can also have other functions. As already briefly mentioned, it can determine the targeting pathway to the membrane, or it may function as a permanent transmembrane anchor. Each of these functions is probably dictated by subtle, yet specific sequence motifs that are still unknown.

Cytosolic Chaperones and Guanosine Triphosphatases

Most membrane systems can only transport proteins that are at least partly unfolded (*15*). Some small proteins (such as the coat protein of phage M13) can spontaneously maintain a loose conformation in the cytosol and travel from the ribosome to the target membrane unassisted (*8*), but large proteins, particularly those with hydrophobic internal domains or hydrophobic signal sequences, require the assistance of cytosolic chaperones or similar factors (*16*). Interaction with a membrane receptor then releases the cytosolic factor and directs the protein's signal sequence into the translocation channel. This release reaction is sometimes controlled by the hydrolysis of ATP or guanosine triphosphate (GTP) (Fig. 3). A few proteins with signal sequences may fully fold in the cytosol and then be unfolded by the membrane-linked protein transport system (*17*).

Chaperones are proteins that interact with nonnative conformations of other proteins. By this interaction they prevent aggregation of newly synthesized proteins until these have either folded correctly in the cytosol or have been transferred to a membrane receptor. Some cytosolic chaperones interact mainly with nascent polypeptides, whereas others interact mainly with proteins that have been released from a ribosome. Examples of factors binding to nascent chains are nascent chain–associated complex (*18*) and trigger factor (*19*); examples of factors binding to released polypeptides are SecB (*7*) and mitochondrial import-stimulating factor (MSF) (*20*). Chaperones also differ in their substrate specificity. Thus, 70-kD heat shock proteins (Hsp70s) interact with a wide spectrum of nonnative proteins, whereas other chaperones, such as SecB and MSF, only interact with proteins destined for transport into or across a membrane. Finally, chaperones also differ with respect to partner proteins and cofactors. Release of a bound protein from SecB only requires interaction with the appropriate acceptor, SecA, but efficient release from Hsp70 or DnaK requires interaction with the co-chaperones DnaJ or GrpE as well as ATP hydrolysis by the chaperone. An even higher level of complexity and regulation is represented by a family of cytosolic ribonucleoproteins termed signal recognition particle (SRP) whose function is regulated by binding and hydrolysis of GTP.

In *E. coli*, there exist at least two pathways for targeting proteins to the export machinery in the plasma membrane. One of these pathways is mediated by SecB and SecA. SecB is a chaperone that has so far only been found in prokaryotes and is dedicated to protein export. SecB binds to basic clusters and apolar regions of nonnative proteins in an ATP-independent manner and delivers these proteins to membrane-bound SecA (*7*, *21*). The second targeting pathway is mediated by SRP and FtsY (*22*). *Escherichia coli* SRP is a cytosolic ribonucleoprotein composed of a 4.5S RNA and a single protein termed fifty-four homolog (Ffh) because it is homologous to the 54-kD subunit of eukaryotic SRP. FtsY is a soluble protein that is homologous to the α subunit of the SRP receptor on the endoplasmic reticulum (see below). Both the SRP subunit and FtsY are guanosine triphosphatases (GTPases). The bacterial SRP binds to the signal sequence of a nascent secretory protein, and the resulting nascent chain–ribosome–SRP complex then binds to FtsY in a GTP-dependent manner. SRP and FtsY increase the targeting efficiency of a subset of *E. coli* secretory proteins, but how they achieve this is not clear. Each of these two bacterial targeting pathways mediates export of only a subset of presecretory proteins. The choice of the pathway is probably governed by a precursor's signal sequence, by sequence motifs within the mature region of the precursor, and by the competition between folding and binding to SecB.

SRP-dependent and -independent targeting is also found with proteins destined for the endoplasmic reticulum (*10*). SRP-dependent translocation in eukaryotes occurs cotranslationally. The coupling between translation and translocation may be the reason why eukaryotic SRP is more complex than its prokaryotic counterpart. Eukaryotic SRP contains a 7S RNA and six protein subunits, one of which (termed SRP54) is a GTPase. SRP binds both to the ribosome as well as to the signal sequence of a nascent protein destined for the endoplasmic reticulum. The specificity of this interaction depends on the ribosome-associated heterodimeric nascent chain–associated complex, which preferentially binds to signal sequence–free nascent chains and prevents them from binding to SRP (*18*); upon binding to a signal sequence and the ribosome, SRP binds GTP and arrests further elongation until the nascent chain–ribosome–SRP complex docks onto the SRP receptor (also termed docking protein) on the endoplasmic reticulum. The SRP receptor is a heterodimer composed of two GTP-binding proteins: a membrane-integrated β subunit and a peripherally attached α subunit. Binding of SRP to its receptor triggers GTP hydrolysis on both SRP and on the SRP receptor, allows release of the signal sequence from SRP, and initiates interaction of the signal sequence with the translocation channel. This GTPase cascade ensures a tight temporal and spatial coupling between translation and membrane translocation: The growing chain passes directly from the ribosome into the translocation channel (cotranslational translocation) (*23*, *24*). A GTP-binding SRP54 also participates in protein transport from the chloroplast stroma into thylakoids (*25*).

Proteins can also be targeted to the endoplasmic reticulum by an SRP-independent, posttranslational pathway that has been characterized best in the yeast *Saccharomyces cerevisiae*. This pathway seems to use cytosolic Hsp70 and the DnaJ homolog Ydj1p (*10*, *26*). Cytosolic Hsp70s (probably together with cytosolic DnaJs) also help to target some proteins to mitochondria and other organelles (*16*), but in no case has the specific membrane receptor been identified.

In addition to the Hsp70-Ydj1p system, mitochondria also use a dedicated, ATP-controlled targeting system for importing some of their proteins. The cytosolic chaperone mitochondrial import-stimulating factor (MSF), a heterodimer composed of a 30- and a 32-kD subunit, specifically binds mitochondrial signal sequences and thereby prevents or even reverses aggregation of mitochondrial precursor proteins in the cytosol

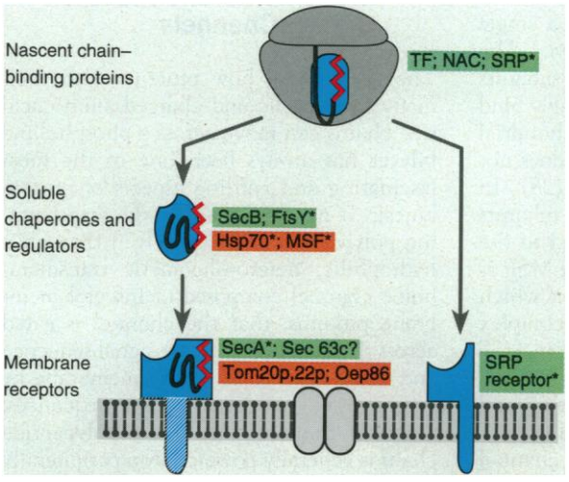

Fig. 3. Protein-targeting pathways. Proteins are targeted to a membrane by different pathways, some of which are regulated by ATP or GTP. Green indicates export system proteins and red-brown, import system proteins. Chaperones, regulatory proteins, or receptors can act at the ribosome, in the cytosol, or on the target membrane. Examples are given for each of these steps. Components that are regulated by nucleoside triphosphate (NTP) are marked by an asterisk. Red zigzag line, signal sequence; NAC, nascent chain–associated complex (*18*); TF, trigger factor (*19*); MSF, mitochondrial import-stimulating factor (*20*).

(*20*). Binding of a mitochondrial precursor protein triggers ATP hydrolysis by MSF. The MSF-precursor complex specifically docks onto the Tom37p-Tom70p receptor on the mitochondrial surface (see below) and releases the precursor to the import channel in the mitochondrial outer membrane.

Membrane Receptors

Protein-translocating membranes of eukaryotes contain receptors that recognize either signal sequences or precursors bound to a cytosolic factor. The known receptors are integral membrane proteins with cytosolically exposed domains. Receptor function can be regulated by the binding and hydrolysis of nucleoside triphosphate. Receptors are usually not fixed to the translocation channel but interact with it dynamically. Deletion of a single receptor is often not lethal and inhibits transport of different precursors to different extents, perhaps because a given precursor may be presented to a membrane system by different routes. The multiplicity of receptors reflects the multiplicity of cytosolic targeting systems and allows a membrane to regulate the targeting of different precursors differentially.

A major protein translocation receptor on the endoplasmic reticulum is the SRP receptor discussed above. Membrane receptors for SRP-independent protein transport into the endoplasmic reticulum (*26*) have not yet been identified, but candidates for such a receptor are subunits of the Sec63 complex, or those of the gated transmembrane channel itself (*24, 26*).

Mitochondria from *S. cerevisiae* and *Neurospora crassa* contain three integral outer membrane proteins that function as protein import receptors. These proteins are termed Tom20p, Tom22p, and Tom70p, according to their apparent molecular masses (*27*). *Saccharomyces cerevisiae* also contains a fourth receptor protein, Tom37p. The four yeast proteins are usually isolated as a Tom37p-Tom70p heterodimer and a less well defined Tom20p-Tom22p subcomplex, but they appear to exist as a single hetero-oligomeric receptor in vivo. The highly acidic Tom20p-Tom22p subunits (termed acid bristle subunits) probably bind the basic and amphiphilic mitochondrial signal sequences. This interaction does not require nucleoside triphosphates (*28*). In contrast, the Tom37p-Tom70p subunits recognize precursors that are bound to the ATP-requiring cytosolic chaperone MSF as discussed above (*20*). It is not clear which feature in the precursor-MSF complex Tom37p-Tom70p recognizes, but free MSF is not recognized. The precursor's signal sequence is then transferred to the acid bristle subunits Tom20p-Tom22p and from there across the protein transport channel in the outer membrane. As with SRP-dependent and -independent translocation into the endoplasmic reticulum, the two mitochondrial receptor subcomplexes can partly assume each other's function. If Tom20p, Tom37p, and Tom70p are deleted, either singly or pairwise, protein import into mitochondria is only partially inhibited and the cells remain viable. Only Tom22p is essential for protein import and viability, presumably because this subunit is also a component of the transport system across the outer membrane (see below). So far, there is no evidence that GTP has a role in mitochondrial protein import.

The binding of precursors to the chloroplast surface appears to be controlled by GTP. The two putative protein import receptors of chloroplasts, Oep34 and Oep86 (for outer envelope protein; also termed Iap34 and Iap86) have GTP-binding motifs, and the binding of precursors to chloroplasts is sensitive to guanosine 5′-*O*-(3-thiotriphosphate) (GTP-γ-S) (*29, 30*). It is not yet known whether these two putative receptor subunits interact with different precursors or whether they recognize signal sequences rather than precursors bound to cytosolic chaperones. In *E. coli*, precursors or precursor-SecB complexes bind directly to the SecA subunit of the translocation machinery. SecA recognizes the precursor's signal sequence, its unfolded mature regions, and SecB (*21, 31*).

When the endoplasmic reticulum or the outer membranes of mitochondria and chloroplasts are solubilized with nonionic detergents, channels and receptors usually do not cofractionate (*10, 27*), suggesting that their interaction in vivo is dynamic. A channel may thus be served by several different receptors. Dynamic interaction of receptors with import channels probably enhances the capture of precursors by translocation channels in a similar way as the excess of different antenna pigments enhances the capture of photons by photosystems.

Transmembrane Protein Channels

The question of how proteins with their many hydrophilic and charged amino acid side chains can move across a phospholipid bilayer has always been one of the most fascinating and baffling aspects of protein kinesis. It now appears that the translocating polypeptide chain is moved through a hydrophilic, hetero-oligomeric transmembrane channel composed of integral membrane proteins; that the channel is gated across the membrane by the signal sequence and within the plane of the membrane by stop-transfer or signal-anchor sequences; and that translocation of the polypeptide chain is generally powered by a peripherally attached protein translocation motor hydrolyzing nucleoside triphosphate. In bacteria and mitochondria, these motors are aided by a transmembrane electrochemical potential (*2, 21*). Protein transport into thylakoids appears to be an exception as import of some proteins can be driven by a pH gradient alone (*32*).

How a protein enters and moves through a channel is not understood. The path of a nascent polypeptide through the endoplasmic reticulum channel has been traced with the aid of polypeptides that were tagged at defined sites with light-activated cross-linkers or environment-sensitive fluorescent probes. The cross-linkers showed that the Sec61 complex is the channel, and fluorescent probes showed that the channel interior is hydrophilic (*23, 33*). The signal sequence was found to be in contact with Sec61 subunits as well as with the membrane lipids, explaining why it is retained on the membrane. The protein-translocating channels of *E. coli* and yeast were also characterized by powerful genetic methods and by reconstituting purified protein fractions into liposomes (*21, 34*).

In the *E. coli* plasma membrane, the transmembrane channel (the first protein transport system to be elucidated in molecular detail) is a heterotrimer composed of the integral proteins SecY, SecE, and SecG. This complex, termed SecYEG, acts in concert with SecA and the membrane proteins SecD and SecF (*21, 31*). The three subunits of the protein channel across the endoplasmic reticulum of mammals and yeast, the Sec61 complex, are similar in sequence to the three subunits of the bacterial SecYEG complex (*35*). In the mitochondrial inner membrane, the protein import channel appears to contain the two integral membrane proteins Tim23 and Tim17 (*36*) and perhaps two additional subunits (*37*). Studies in chloroplasts have implicated two integral proteins of the inner envelope (Iap100 and Iap36) as candidate components of the protein transport channel across that membrane (*29*).

Mitochondria and chloroplasts also have export systems. The mitochondrial system has not yet been properly characterized, but it seems to insert mitochondrially encoded proteins into the inner membrane (*2*). There is no evidence that it exports proteins into the soluble intermembrane space. The chloroplast system is better characterized and also more versatile: It acts not only on chloroplast-encoded proteins but also on imported proteins and transports them into the thylakoid membrane, the thylakoid lumen, and perhaps also into the inner envelope membrane (*38*). As chloroplasts contain homologs of bacterial SecA and SecY (*39*) as well as SRP54 (*25*), their export system closely resembles that of bacteria.

As protein-transporting membranes generally do not allow the passive diffusion of ions, the protein transport channels must open up only in response to a translocating polypeptide. Several lines of indirect evidence suggest that such a gating across the membrane may be controlled by the appropriate signal sequence. First, mutations in a signal sequence for the bacterial plasma membrane can be suppressed by mutations in the channel subunit SecY, indicating a specific interaction between these two partners (*40*). Second, the Sec61 channel of the mammalian endoplasmic reticulum effectively discriminates between functional and nonfunctional signal sequences (*24*). Third, electrophysiological studies have detected large-conductance channels in the endoplasmic reticulum and the *E. coli* plasma membrane that are specifically blocked by a translocating polypeptide chain or opened by a chemically synthesized signal peptide (*41*). However, it is not yet certain that these gated ion channels are indeed protein transport channels. As already mentioned, protein-translocation channels are also gated within the plane of the membrane, releasing hydrophobic stop-transfer sequences into the lipid bilayer. Some steps of this lateral release might well be the mirror image of the steps by which hydrophobic export signal sequences interact with the channel: The hydrophobic segment of the signal sequence might first partition into the hydrophobic core of the bilayer and then enter the transport channel laterally.

Protein transport channels are not active transporters but conduits that functionally resemble the F_0 portion of the proton-translocating F_1F_0–adenosine triphosphatase (ATPase). In the F_1F_0-ATPase, active proton transport is effected by coupling the gated proton channel F_0 to the peripheral proton pump F_1, which is powered by the hydrolysis of ATP (*42*). In transmembrane protein transport, most protein channels are coupled to a peripheral protein translocation motor that is powered by the hydrolysis of GTP or ATP and that either pushes or pulls the protein across the membrane.

There exist at least three types of membrane-associated protein translocation motors (Fig. 4). One is Hsp70, which is bound to the trans side of the membrane. This motor provides an ATP-powered "pull" and effects posttranslational import into the endoplasmic reticulum, mitochondria, and probably also into chloroplasts. Another motor is SecA that is bound to the cis side of the membrane. This motor provides an ATP-powered push and effects protein transport across the bacterial plasma membrane and probably also the thylakoid membrane. A third type of motor might be a ribosome that is bound to the cis side of the membrane. In principle, this motor could provide a GTP-powered push, but there is currently no compelling proof that ribosomes can push a growing polypeptide chain against a resistance.

The ATP-powered pull by a member of the Hsp70 family has been studied in the endoplasmic reticulum of yeast and in mitochondria. In the endoplasmic reticulum of yeast, a Hsp70 (termed Kar2p or BiP) is bound to a lumenally exposed DnaJ-like domain of Sec63p, a transmembrane subunit of the hetero-oligomeric Sec63 complex (*43*). A similar arrangement is found in yeast mitochondria: Import across the inner membrane is mediated by a matrix-located Hsp70 (termed mHsp70), which is bound to the cochaperone GrpEp and to the inner membrane protein Tim44p. The mHsp70-GrpEp-Tim44p complex interacts loosely with the import channel in the inner membrane (*44*). In both cases, the Hsp70 on the trans side of the membrane interacts with the translocating polypeptide chain and, by hydrolyzing ATP, pulls it across the membrane.

How does such an ATP-driven pull work? The molecular ratchet model (Fig. 5) proposes that Hsp70 binds to segments of the polypeptide as these emerge on the trans side of the membrane by random oscillation within the transport channel (*45*). Acting like Maxwell's demon, Hsp70 thus converts a random Brownian motion into a unidirectional movement. According to this passive capture model, the rate of posttranslational transport of proteins with tightly folded domains should be limited by the spontaneous unfolding of such domains on the cis side of the membrane barrier. However, mitochondria can rapidly import precursor proteins with tightly folded domains whose spontaneous unfolding would require hours (*17*). This predicament has spawned the translocation motor model (*46*) (Fig. 5). This model proposes that the translocating polypeptide binds to Hsp70, that this binding stimulates ATP hydrolysis by Hsp70, and that ATP hydrolysis causes a conformational change in Hsp70, which actively pulls a

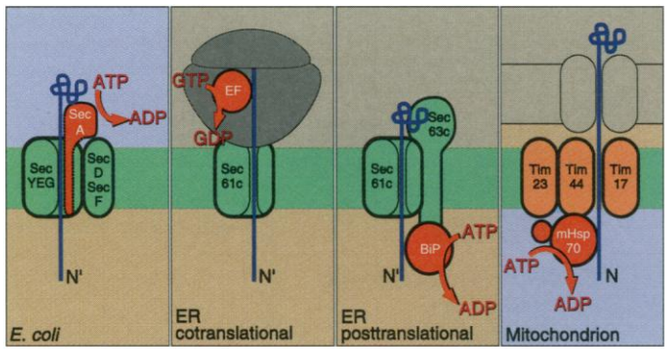

Fig. 4. The protein translocation systems of the endoplasmic reticulum, the mitochondrial inner membrane, and the bacterial plasma membrane. Export systems are in green, import systems in red-brown, NTP-coupled protein translocation motors in red, cytosolic spaces in light blue or gray, and extracytosolic spaces in tan. The two systems of the endoplasmic reticulum represent the posttranslational system identified in yeast and the cotranslational system. EF, ribosome-associated elongation factor of protein synthesis; Sec63c and Sec61c stand for the respective hetero-oligomeric complexes; Tim, transport components of the mitochondrial inner membrane. BiP (Hsp70) may also be required for cotranslational translocation into the endoplasmic reticulum (*50*). The SecA domain surrounded by a dotted line is involved in the insertion-deinsertion cycle during the motor function of this protein. N′ indicates an NH_2-terminus generated by proteolytic removal of the presequence.

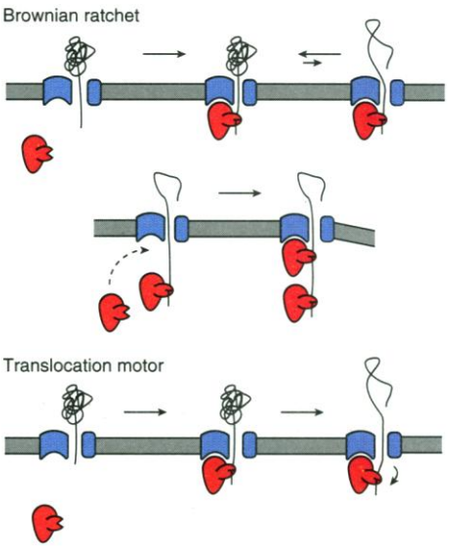

Fig. 5. Two models for the function of Hsp70 as an ATP-driven protein translocation motor. (**A**) The molecular ratchet model proposes that Hsp70 (red) binds to the translocating chain as it emerges on the trans side (bottom) of the membrane barrier as a result of random oscillation within the translocation channel (blue). By preventing back-movement, successive rounds of Hsp70 binding convert a random into a unidirectional motion. This model predicts that the rate of translocation of folded proteins is determined by the rate of spontaneous unfolding. (**B**) The translocation motor model proposes that Hsp70 captures the incoming chain and then undergoes an ATP-driven conformational change that actively pulls a segment of the bound chain across the membrane. This model implies that Hsp70 can actively unfold precursors. Adapted from (*46*).

segment of the bound chain across the membrane. By generating an inward force, Hsp70 can thus act as an unfoldase for a protein domain that has not yet crossed the membrane. Finally, GrpEp-triggered adenosine diphosphate–ATP exchange on mHsp70 would restart the cycle with a downstream segment of the polypeptide chain. The translocation motor model invokes a functional analogy to the myosin-actin interaction in muscle contraction. These two models are not mutually exclusive. Studies with yeast mitochondria containing mutant mHsp70 proteins are compatible with the possibility that the molecular ratchet mode operates with loosely folded precursors, whereas the translocation motor mode is called up for the translocation of tightly folded protein domains (*47*).

The ATP-powered push by SecA in bacterial protein export is the best documented example of an ATP-driven protein translocation motor (*48*). SecA does not resemble Hsp70 proteins structurally but, like Hsp70s, is an ATPase whose activity is stimulated by unfolded polypeptide chains. Unlike Hsp70s, SecA specifically binds the cytosolic chaperone SecB as well as acidic phospholipids that are essential for maximal ATPase activity. SecA is attached to the cytosolic surface of the plasma membrane through acidic phospholipids and the SecYEG complex. Upon binding ATP, SecA interacts with the polypeptide destined for export and then undergoes a massive conformational change that pushes a 30-kD SecA domain all the way across the plasma membrane. This transmembrane movement of a SecA domain carries a segment of the translocating polypeptide chain across the membrane. Hydrolysis of the bound ATP induces return of the translocated SecA domain to its ground state on the cytosolic face of the plasma membrane and releases the translocating polypeptide from SecA. The released chain is then translocated further by the transmembrane electrochemical potential until it is recaptured by the next round of the SecA cycle. The SecA motor thus has two power strokes, one fueled by ATP and the other by a transmembrane potential. Why do bacteria push whereas the endoplasmic reticulum and mitochondria pull? A reason may be the lack of ATP on the trans side of the bacterial plasma membrane: Bacteria have put the motor where the fuel is.

Whether a translocating polypeptide chain can be pushed across a membrane by a membrane-bound ribosome is still unproven. If such a push exists, it can only be exerted by the GTP-powered polypeptide elongation machinery that extrudes the growing polypeptide chain from the ribosomal tunnel across the transmembrane protein channel (*6*). In order for this mechanism to work, the ribosome must be fixed to the membrane independently of the translocating polypeptide chain. This condition is met. The ribosome is tightly bound to the endoplasmic reticulum through specific, salt-sensitive interactions with the Sec61 complex and perhaps additional "ribosome receptors" (*24*, *49*). However, the situation may be more complex because recent data suggest that Hsp70 (BiP) is required even for cotranslational protein translocation (*50*): BiP was found to be essential for cotranslational transport of invertase into yeast microsomes. This BiP requirement was not observed when transport was assayed in a microsomal system that had been reconstituted from purified components. This discrepancy is unexplained; the ribosomal push may need the help of an Hsp70-mediated pull, or Hsp70 may be needed to remove the translocated chain from the exit site of the translocation channel.

The outer membranes of mitochondria and chloroplasts pose special problems because they lack ATP-driven protein translocation motors or a significant transmembrane potential. Yet most of the organellar proteins must be completely transported across the outer membranes to reach internal compartments. Although it is not entirely clear how transport across the outer membranes is energized, preliminary evidence suggests that the precursor's signal sequence is driven across the membrane by association with one or more binding sites on the inner side of the outer membrane (*51*). In the yeast mitochondrial outer membrane, one of the trans binding sites may be contributed by the import receptor Tom22p, which has a small acidic domain that protrudes into the intermembrane space and has a high affinity for mitochondrial signal sequences. In chloroplasts, a functionally equivalent site may be provided by a Hsp70 that is bound to the inner face of the outer membrane. Binding to this Hsp70 may explain why binding of precursors to the chloroplast surface requires low concentrations of ATP in the intermembrane space (*52*). The signal sequence may thus be transported across the outer membranes of mitochondria and chloroplasts by a relay of binding sites on both faces of the membrane. A similar binding cascade is invoked for the movement of proteins through the nuclear pore complex (*4*). Once the signal sequence of a protein destined for internal compartments has emerged in the intermembrane space, it may be captured by the inner membrane system whose ATP-linked protein translocation motor completes translocation. In mitochondria, capture by the inner membrane system is initiated by the electric potential

Table 1. Proteins of export and import systems. Proteins with similar functions share the same horizontal line, proteins with similar sequence the same color. Mammalian homologs of yeast proteins are in parenthesis. See Fig. 1 for abbreviations and color coding.

	Export systems			Import systems		
	E. coli	Endoplasmic reticulum	Chloroplast (thylakoid)	Mitochondrion	Chloroplast	Peroxisome
Cytosolic chaperones or regulators	Trigger factor; SecB DnaK SRP-GTP FtsY-GTP	NAC Hsp70 SRP-GTP	Hsp70 SRP-GTP	Hsp70 MSF-ATP	Hsp70	Pas10p (Pas 8p); Pas7p
Membrane receptors	SecA	SRα, SRβ-GTP	SecA	Tom20p-Tom22p Tom37p-Tom70p	Oep86, 34-GTP	Pas20p
Channels	SecY SecG SecE SecD, SecF	Sec61p (Sec61α) Sbh1p (Sec61β) Sss1p (Sec61γ) Sec63p, 62p, 71p, 72p	SecY	Tim17p, 23p Tim44p	Iap100, 36 ?	
Translocation motors	SecA	Ribosome Kar2p (BiP)	SecA	mHsp70	chHsp70	?
		Outer membrane channels of organelles		Tom40p, 6p, 7p, 8p	Oep (Iap) 75	?

across that membrane that electrophoretically pulls the positively charged signal sequence across the inner membrane (*53*).

Protein translocation across different membranes is thus effected by a limited set of machines composed of functionally analogous sets of components. However, most components of the export systems are structurally quite different from their functional counterparts in the import systems (Table 1). The only exceptions are the members of the Hsp70 family that participate in both types of systems, but Hsp70s participate in many different aspects of protein folding and are not dedicated to protein transport across membranes. All export systems share some highly conserved components such as SRP, SRP receptor (FtsY), or the subunits of the translocation channel. In contrast, the membrane-embedded proteins of the import systems of mitochondria and chloroplasts are not only different from each other but also from all known proteins. These import machines might have evolved from as yet undiscovered peptide or metabolite transporters of prokaryotic ancestors. Indeed, the protein channel subunit Tom40p of the outer membrane of fungal mitochondria has a predicted β sheet structure that is typical of pore-forming proteins of bacterial outer membranes, although even in this case the primary sequence of the mitochondrial protein is completely different from those of its bacterial counterparts (*54*).

Chaperones and Folding Catalysts

As most membranes can only transport loosely folded proteins, exit of a protein from the transport channel is followed by folding on the trans side of the membrane. Efficient folding is usually mediated by a battery of chaperones and other folding helpers (*16*). Most of these can interact with a wide spectrum of different nonnative proteins, but some act on only a few, or even a single protein. Folding may require the successive action of several chaperones or folding catalysts.

The chloroplast stroma and the mitochondrial matrix contain homologs of bacterial GroEL and GroES (termed Hsp60 and Hsp10, or Cpn60 and Cpn10) that facilitate the folding of imported as well as organelle-synthesized proteins. It was first thought that these organellar chaperonins are required for the folding of all imported proteins, but it is now clear that they are only required for a subset of them. In mitochondria, several monomeric proteins with simple folding pathways fold rapidly without the help of Hsp60; some fold by means of an intramitochondrial DnaJ or a cyclosporin-sensitive proline rotamase, and others may fold by means of a subfraction of mHsp70 that is soluble in the matrix (*55*). This soluble mHsp70 also acts in quality control by binding misfolded proteins and delivering them to the mitochondrial Lon protease for degradation (*56*).

Refolding of exported bacterial proteins in the periplasm is mediated by several chaperones with very narrow substrate specificity, by disulfide isomerases, and by at least one proline rotamase (*57*). The endoplasmic reticulum lumen contains several well-characterized chaperones such as BiP, Hsp90, and calnexin, which cooperate with each other and which probably all facilitate the folding of imported proteins. However, a role in protein folding has so far only been clearly established for BiP (*58*), disulfide isomerase, and proline rotamases (*57*). BiP and calnexin also act as quality controls, allowing only properly folded proteins to be transported along the secretory pathway. Improperly folded proteins are retained in the endoplasmic reticulum and eventually targeted for proteolytic degradation (*58*). Some chaperones, such as mHsp70 or BiP, thus perform at least three different functions in protein transport across membranes: translocation, folding, and quality control. It is usually difficult to assay one of these functions independently of the others.

And Peroxisomes?

Most proteins destined to be imported into peroxisomes carry a COOH-terminal Ser-Lys-Leu (or closely similar) tripeptide motif collectively termed PTS1 (for peroxisome-targeting signal). A few other imported proteins carry a transient NH_2-terminal signal (termed PTS2) with a loose 11-residue consensus motif, and still others contain unidentified signals (*59*). PTS1 signals are recognized by a protein termed Pas8p or Pas10p (depending on the yeast species), but there is no agreement on whether this protein is located in the cytosol or on the peroxisome membrane. PTS2 signals are recognized by the protein Pas7p, but again there is disagreement on whether this protein is in the cytosol or in the peroxisomal matrix. Pas8p (or its homolog Pas10p) appears to be recognized by the peroxisomal integral membrane protein Pas20p (*60*). Peroxisomes may thus resemble the other membrane systems in using a cytosolic-targeting system coupled to a membrane receptor, but no channel subunits have yet been identified. We also do not know how protein transport into peroxisomes is energized, how peroxisomes can import large folded proteins, and whether proteins are imported into unidentified peroxisomal precursor structures in vivo. Peroxisomes may well blend into the general picture once their translocation system is better understood, but at the moment they are difficult to integrate into a unified view of membrane-linked protein transport.

Outlook

In this article we have depicted protein translocation across membranes with a broad brush, de-emphasizing details and minor exceptions in order to highlight common principles. The uniformity of basic mechanisms that has emerged from recent work places protein translocation firmly within the framework of other membrane-linked transport systems. Of course, some of the principles described here may not stand the test of time. It is still prudent to seek simplicity, and then to distrust it.

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Coat Proteins and Vesicle Budding

Randy Schekman* and Lelio Orci

The trafficking of proteins within eukaryotic cells is achieved by the capture of cargo and targeting molecules into vesicles that bud from a donor membrane and deliver their contents to a receiving compartment. This process is bidirectional and may involve multiple organelles within a cell. Distinct coat proteins mediate each budding event, serving both to shape the transport vesicle and to select by direct or indirect interaction the desired set of cargo molecules. Secretion, which has been viewed as a default pathway, may require sorting and packaging signals on transported molecules to ensure their rapid delivery to the cell surface.

Eukaryotic cells have an elaborate network of organelles, many of which are in constant and bidirectional communication through a flow of small transport vesicles. For each organelle a specific mechanism exists to capture and package certain proteins and lipids that are destined for transport to a receiving compartment. In return, the receiving compartment accepts proteins that are meant to remain, or to be passed to another station, and then retrieves for recycling other proteins that belong in the donor organelle. Among the recycled proteins are structural components of the traffic pathway that must be used repeatedly to sustain transport. The most remarkable feature of this process is that selectivity is achieved in spite of the fluid nature of the membrane. In the absence of specific mechanisms to recognize and sequester proteins destined for transport and retrieval, communicating organelles would quickly lose their identity, succumbing to the lateral diffusional mobility of membrane proteins embedded within the bilayer. The evidence that we summarize in this review suggests that membrane identity is maintained by the selective capture into coated vesicles of proteins destined for transport.

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Three Paradigms of Vesicle Bud Formation

Three models have contributed to our understanding of the mechanism of vesicle budding. The first is fashioned on the example of enveloped viruses that bud from