Delivering proteins for export from the cytosol

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Abstract | Correct protein function depends on delivery to the appropriate cellular or subcellular compartment. Following the initiation of protein synthesis in the cytosol, many bacterial and eukaryotic proteins must be integrated into or transported across a membrane to reach their site of function. Whereas in the post-translational delivery pathway ATP-dependent factors bind to completed polypeptides and chaperone them until membrane translocation is initiated, a GTP-dependent co-translational pathway operates to couple ongoing protein synthesis to membrane transport. These distinct pathways provide different solutions for the maintenance of proteins in a state that is competent for membrane translocation and their delivery for export from the cytosol.

The transport and trafficking of newly synthesized polypeptides in cells allows for the generation and maintenance of distinct environments that are crucial for different cellular functions. In most organisms, to reach the correct location, a high proportion of proteins must cross at least one biological membrane. The mechanisms for this protein delivery have been subject to strict evolutionary control. These mechanisms can be divided into two fundamentally distinct processes: the transport of polypeptides from the cytosol to an extracytosolic compartment (called protein export) and the transport of proteins into compartments that are functionally equivalent to, or evolutionarily derived from, the cytosol (called protein import) (FIG. 1). By these criteria, the process of protein export excludes the transport of proteins to mitochondria, peroxisomes and plastids, and is exemplified by the delivery of precursors to the inner membrane of prokaryotes and the endoplasmic reticulum (ER) of eukaryotes.

Both prokaryotic and eukaryotic cells must coordinate the delivery of several distinct classes of protein substrate to their final destination. Secretory proteins must be first delivered to, and then entirely translocated across, the export membrane, whereas the complete translocation of membrane proteins is prevented to facilitate their integration into the lipid bilayer. Nevertheless, the delivery processes for these different classes of substrate largely overlap, and two major modes of delivery are found in both prokaryotes and eukaryotes. Protein delivery can occur by a post-translational mechanism after the completion of synthesis, or, alternatively, the nascent polypeptide can be delivered to the export membrane together with the ribosome during protein synthesis by a co-translational delivery mechanism. It is suggested that co-translational delivery evolved after the post-translational routes, and would seem to offer a more sophisticated solution to protein delivery. Hence, co-translational delivery avoids many of the potential complications that are encountered during post-translational delivery, such as those caused by the synthesis of complex folding domains, and seems to be better suited to the delivery of membrane proteins.

The export of proteins from the cytosol can be divided into three key steps: substrate recognition, delivery to the destination membrane and translocation across or into a membrane. The study of protein export has uncovered numerous pathways and components that control each of these steps, and it seems that individual substrates might exploit distinct combinations of the available pathways during their export. During substrate recognition, the precise choice of delivery pathway made by each protein is largely determined by the presence and location of specific signal sequences in the newly synthesized polypeptide. These export sequences minimally comprise a span of hydrophobic amino acid residues and direct precursors to the export site by binding to specific cytosolic factors. For secretory proteins, this signal sequence is usually at the amino terminus and is cleaved once the protein has crossed the membrane. Membrane proteins might have a similar cleavable N-terminal signal or might instead be directed to the membrane by their hydrophobic transmembrane-spanning region. The role of a hydrophobic signal sequence in directing precursor proteins...
SecB binds with high affinity only to SecA that is associated with the SecYEG channel, ensuring efficient delivery to the export site (see reviews). On interaction with SecB, SecA binds both to the signal sequence and elsewhere in the substrate protein, which helps to ensure the specificity of protein export. SecA then pushes the substrate stepwise through the adjacent SecYEG translocon. This is achieved through conformational changes of SecA that are coupled to its ATPase cycle. Recent structural analysis of SecA bound to the SecYEG complex suggests a mechanism for this process. It seems that a distinct domain in SecA, termed the two helix finger, protrudes into the translocation channel and might act as a translocation piston to push the substrate into the periplasmic space. A Tyr residue at the tip of this finger seems to be essential for this activity, and might directly contact the substrate as the SecA ATPase drives several rounds of piston insertion. In addition to its activity at the translocon, it has also been suggested that a distinct cytosolic pool of SecA might promote membrane delivery by directly associating with the signal sequence of nascent secretory proteins independently of SecB. The DNAK chaperone is thought to bind to the secretory precursor and prolong its translocation competence, thereby increasing the timeframe available for successful engagement of the signal sequence with the export channel. A similar effect has also been suggested for the HSP60 complex chaperonins GroEL and GroES. In the absence of a receptor for these ubiquitous chaperones at the export site, the delivery of the polypeptide is probably driven by the binding affinity of the signal sequence for the SecYEG channel itself.

DnaK might also have a role in another post-translational export pathway that occurs through the so-called Tat (twin Arg translocation) pathway, a distinct export route to that provided by SecYEG that is exploited by a special class of proteins. Substrates of this pathway are characterized by a twin Arg motif present in their N-terminal export signal. The Tat pathway mediates the export of tightly folded proteins, in particular cofactor-containing redox enzymes, and interactions between these substrates and DnaK probably promote export by sheltering the signal sequence from premature engagement with the TatABC translocon. Alternatively, the twin Arg motif can also be recognized by specialized chaperones, such as SlyD, that might coordinate precursor folding and subsequent delivery to the TatABC translocon (FIG. 2).
Eukaryotic post-translational delivery

In eukaryotic cells, a distinct class of substances termed the tail-anchored (TA) proteins are delivered to the ER membrane by a strictly post-translational mechanism. These membrane proteins contain a single carboxy-terminal signal sequence that is only exposed to cytosolic targeting factors on termination of protein synthesis and release from the ribosomal exit tunnel. Hence, the position of the signal sequence in the polypeptide chain dictates that the subsequent delivery step is post-translational. Most TA protein delivery to the ER membrane is dependent on ATP hydrolysis. However, it has recently become clear that there are two distinct ATP-dependent delivery routes to the ER for TA proteins. The first pathway requires that the HSP40–HSP70 chaperone complex binds to the ER membrane, probably acting to prevent the misfolding and aggregation of the newly synthesized precursors. The second pathway is mediated by the cytosolic ATPase ASNA1. ASNA1 binds to the signal sequence of TA proteins after synthesis is completed and, like HSP40–HSP70, maintains the competence of translocation. It might also be that the ASNA1 pathway provides a specialized delivery route that is responsive to particular environmental challenges, such as oxidative stress. In *Saccharomyces cerevisiae*, a dedicated receptor for the yeast ASNA1 homologue, Golgi to ER traffic protein 3 (Get3), has been identified and forms a complex with Get1 and Get2 at the ER membrane. Loss of this receptor results in the formation of cytosolic Get3–TA protein aggregates and prevents the delivery of TA proteins to the ER. Thus, whereas the precise mechanism for signal sequence recognition and binding by Get3 or ASNA1 is unclear, this membrane delivery route is a distinct, multi-component targeting module that is comparable to the SecA–SecB pathway in prokaryotes. By contrast, no such ER membrane receptor has been identified for HSP70-bound TA proteins, and for both pathways the events downstream of initial precursor recognition and binding are poorly understood.

The choice of pathway used by individual TA protein substrates is based on the net hydrophobicity of the signal sequence itself. TA proteins with more hydrophobic signal sequences favour the ASNA1 delivery route, whereas TA proteins with less hydrophobic signals are obligatory clients for the HSP40–HSP70 pathway. However, it seems likely that there is some overlap between these pathways and, similar to the partial redundancy of the SecA–SecB delivery route in prokaryotes, the HSP40–HSP70 route might provide a fall-back delivery mechanism for essential TA proteins in the absence of ASNA1 function. Following TA protein delivery to the ER, the molecular mechanisms that underlie membrane integration are unclear. In particular, any role for the Sec61 translocation complex (BOX 1; see below) is highly controversial.

Members of the eukaryotic HSP40 and HSP70 family are analogous to the prokaryotic DnaJ and DnaK proteins. In addition to their role during TA protein synthesis, these cytosolic chaperones mediate the export of some secretory proteins across the ER membrane. At least one of their functions is to hold a TA protein in an appropriate conformational state that is suitable for efficient membrane translocation and export. However, despite the complexity and diversity of the post-translational delivery routes in eukaryotes, these pathways primarily accommodate specialized groups of precursors, and most eukaryotic export substrates are thought to be delivered by an alternative co-translational mechanism.

Co-translational protein delivery

An alternative to using chaperones to maintain the competence of translocation of proteins destined for export is to couple protein synthesis to membrane translocation, by delivering the nascent polypeptide to the membrane during translation. This co-translational translocation strategy avoids the probability of premature protein folding and exploits the energy of the translation process to drive the polypeptide chain across the export membrane. For integral membrane proteins, co-translational delivery also prevents the potential exposure of multiple hydrophobic transmembrane segments to the aqueous cytosolic environment and ensures their coordinated insertion and assembly at the membrane. Hence, co-translational delivery avoids many of the potential
complications that are associated with post-translational delivery by pre-emitting the exposure of the bulk of the nascent chain to the cytosol.

Co-translational translocation seems to be a universal strategy for efficient protein delivery to the inner membrane of prokaryotes and the ER membrane of eukaryotes. Interestingly, although co-translational delivery is thought to have evolved after many post-translational mechanisms, under normal cellular conditions it might be the dominant mode of delivery of most substrates to the export membrane, particularly in higher eukaryotes. The predominant pathway for co-translational delivery to the export membrane is mediated by the highly conserved SRP and is dependent on GTP. The minimal components for a co-translational, SRP-dependent delivery pathway, such as that found in prokaryotes, are a highly conserved SRP54-like protein (fifty-four homologue (Ffh) in Escherichia coli), bound to a structurally conserved RNA molecule together with a membrane-associated receptor protein. The defining feature of SRP-dependent delivery is the recognition and binding of hydrophobic signal sequences located towards the N terminus of the nascent polypeptide as soon as they emerge from the ribosome. In prokaryotes, this pathway largely favours the delivery of inner membrane proteins, although some secretory proteins also exploit this route. In eukaryotic cells, the SRP pathway delivers most secretory and membrane proteins to the ER. Signal sequence hydrophobicity promotes the use of the SRP-dependent delivery route, although in one example, an inhibitory sequence that overrides this co-translational targeting process has been identified.

Signal sequence recognition by the SRP. The SRP recognizes export signals that lack any precise sequence arrangements. Instead, it discriminates these signals on the basis of their hydrophobicity and secondary structure. The central region of such a signal sequence typically adopts an α-helical structure that is formed in the exit tunnel of the ribosome, before exposure to the SRP. The C-terminal M domain of SRP54 comprises a hydrophobic cleft that is enriched in Met residues, which bind to the central region of export signals and the GTase domain (NG domain) of SRP54 can also contribute to export signal binding.

Although the structures of both prokaryotic and eukaryotic ribosome–nascent chain–SRP complexes have been determined using cryo-electron microscopy, the exact details of signal sequence recognition by SRP have not been resolved. A complication in prokaryotes is the presence of a protein called trigger factor on translating ribosomes. This component has been implicated in aspects of both protein folding and protein delivery to the membrane, and docks at the ribosomal protein L23, which is located near the nascent chain exit site and adjacent to the site of SRP binding to the ribosome. Although the precise interplay between trigger factor and SRP is unresolved, trigger factor might act to modulate co-translational delivery, both by competing with SRP for binding to nascent chains and by masking a ribosomal binding site for the translocon.

Coupling SRP recognition to delivery. Following signal sequence recognition, the ribosome–nascent chain–SRP complex is recruited to the export membrane by its interaction with a dedicated SRP receptor (Fig. 3a,c). In prokaryotes, the role of the SRP receptor is fulfilled by a component called FtsY (Fig. 3), whereas in eukaryotes the SRP receptor (SR) comprises the FtsY homologue together with a second membrane-anchored component, SRβ (Fig. 4).

When SRP interacts with a signal sequence at the ribosome, the conserved SRP core (SRP54 and SRP RNA; Fig. 5a) undergoes substantial structural rearrangements. As a consequence, the SRP54 GTase domain is loaded with GTP and SRP is primed for its subsequent interaction with the SR. The GTase activities of SRP and SR are synchronized by the formation of a nearly symmetrical dimer (the SRP–SR docking complex), which results in a composite active site with both GTases reciprocally activating each other (Fig. 3c). The activation of both GTases in the SRP–SR

M domain
A Met-rich domain of the signal recognition particle SRP54 that both houses the signal sequence recognition region and binds the SRP RNA.

NG domain
A domain in the signal recognition particle SRP54 that comprises a four helix bundle and the GTase domain.

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**Figure 3 | Structural insights into SRP–SR interaction.** a) Domain structure of the signal recognition particle (SRP) SRP54 (fifty-four homologue (Ffh) in *Escherichia coli*) and the SRP receptor (SR) component SRα (FtsY) in *E. coli* (Ec), *Homo sapiens* (Hs) and *Arabidopsis thaliana* chloroplasts (At cp). These GTases are multidomain proteins and share conserved GTase domains (NG domains). The location of two important protein–interaction sites in FtsY is indicated by asterisks. Eukaryotic SRα contains an additional longin domain (X2) at the amino terminus, which binds SRα to SRβ. In cpSRP54, the carboxy-terminal extension contains novel basic residues (indicated by ++) that are essential for cpSRP43 interaction, and the Met-rich M domain is responsible for signal–sequence binding. b) The ribosomal L23 protein in prokaryotes (L25 in eukaryotes; brown) is a conserved platform for multiple interactions, including trigger factor (TF), SRP and the SecYEG or Sec61 translocon, that occupy overlapping binding sites. c) Ffh and FtsY form a highly symmetrical heterodimer in *E. coli* (Ec), *Homo sapiens* (Hs) and *Arabidopsis thaliana* chloroplasts (At cp). These GTases are multidomain proteins and share conserved GTase domains (NG domains). The location of two important protein–interaction sites in FtsY is indicated by asterisks. Eukaryotic SRα contains an additional longin domain (X2) at the amino terminus, which binds SRα to SRβ. In cpSRP54, the carboxy-terminal extension contains novel basic residues (indicated by ++) that are essential for cpSRP43 interaction, and the Met-rich M domain is responsible for signal–sequence binding. d) The ribosomal L23 protein in prokaryotes (L25 in eukaryotes; brown) is a conserved platform for multiple interactions, including trigger factor (TF), SRP and the SecYEG or Sec61 translocon, that occupy overlapping binding sites. e) The ribosomal L23 protein in prokaryotes (L25 in eukaryotes; brown) is a conserved platform for multiple interactions, including trigger factor (TF), SRP and the SecYEG or Sec61 translocon, that occupy overlapping binding sites. f) The ribosomal L23 protein in prokaryotes (L25 in eukaryotes; brown) is a conserved platform for multiple interactions, including trigger factor (TF), SRP and the SecYEG or Sec61 translocon, that occupy overlapping binding sites. g) The ribosomal L23 protein in prokaryotes (L25 in eukaryotes; brown) is a conserved platform for multiple interactions, including trigger factor (TF), SRP and the SecYEG or Sec61 translocon, that occupy overlapping binding sites. h) The ribosomal L23 protein in prokaryotes (L25 in eukaryotes; brown) is a conserved platform for multiple interactions, including trigger factor (TF), SRP and the SecYEG or Sec61 translocon, that occupy overlapping binding sites.
complex has been assigned a checkpoint role in protein translocation and can regulate the delivery process. Hence, mutants that can form this complex but have compromised GTPase activation have defects in protein translocation. The binding of SRP to the ribosome and the docking of the ribosome onto the SecYEG or Sec61 export channel are mutually exclusive events, and the interaction between the SRP and FtsY or SR promotes structural rearrangements in the complex that allow ribosome–nascent chain binding to the SecYEG or Sec61 translocon. Hence, the SRP–SR interaction is required for release of the signal sequence by SRP and subsequent insertion of the export signal into the translocation channel. Last, SRP and SR dissociate on GTP hydrolysis and can then enter into another round of delivery (FIG. 4). Thus, SRP and SR sense and react to the presence of the signal sequence and to the availability of a translocation channel to precisely coordinate the individual steps of co-translational targeting.

What triggers the transfer of the ribosome–nascent chain complex from SRP to the export channel is not well understood. The SRP RNA seems to have an important role and both accelerates the rate of SRP–SR complex formation and stimulates GTP hydrolysis. Formation of the SRP–SR complex triggers a conformational change that localizes the SRP RNA at the interface of the GTPase heterodimer. If this change occurs before the ribosome–nascent chain dissociates from SRP, export signal–dependent perturbations in the SRP RNA might directly inhibit GTP hydrolysis. Thus, after the release of the export signal from SRP, the close proximity between the RNA and the now empty binding site in the M domain of SRP54 could trigger GTP hydrolysis, thereby promoting the final disassembly of the SRP–SR complex.

Interestingly, at steady state, the SRP receptor FtsY is evenly distributed between the cytosol and the inner membrane of E. coli. This observation is probably explained by the absence of a membrane–anchored SR homologue. Thus, FtsY might also interact with SRP that is bound to ribosome–nascent chain complexes in the cytosol and, in this way, soluble FtsY might contribute to protein delivery to the export membrane. However, the presence of the export membrane is a prerequisite for the release of the nascent chain from SRP–FtsY, providing a regulatory step that prevents exposure of the export signal to the cytosol during delivery. Once at the membrane, the interaction between SecYEG and FtsY is thought to announce the availability of a vacant export channel and to prime it for the subsequent interaction of the ribosome–nascent chain–SRP complex. This interaction with FtsY is suggested to be mediated by an extensive cytosolic domain in the translocon subunit SecY and is modulated by GTP binding and hydrolysis.

In addition, FtsY is thought to contact the membrane directly through an amphipathic helix at the N terminus of the FtsY N domain (FIG. 5a) that might form a transient membrane anchor. A second lipid contact is provided by the N terminus of the FtsY A domain (FIG. 3a), and...
although the presence of this region is not essential, it is thought to stabilize the PtsY–SecY interaction and stimulate co-translational targeting\(^8\). This extensive regulation of PtsY probably reflects its convergence with other delivery routes at the export channel (FIG. 2). Given that it is estimated that only ~300 copies of the translocon are present per E. coli cell, the composition of the export site is probably highly dynamic, and SecYEG or Sec61 might recruit additional factors from a pool of many different accessory components in response to the requirements of specific substrates during export (BOX 1).

A potential benefit of co-translational translocation in both prokaryotes and eukaryotes is the enrichment of specific mRNAs at the export site as a consequence of polysome formation, which might act to enhance the efficiency of the delivery of certain precursor proteins\(^9\) (FIG. 4). In this process, as new ribosomes begin translation, the emerging polypeptides are already in close association with the membrane export machinery, potentially preventing the requirement for membrane-targeting processes.

**SRP in post-translational mode.** While the conventional view of SRP is that it acts strictly co-translationally, in vitro experiments have shown that mammalian SRP can maintain certain secretory and membrane proteins in a conformation that is competent for translocation or membrane integration, probably by preventing substrate aggregation. This is akin to the role that is suggested for chaperones of the DnaJ and DnaK family\(^{10,11}\). A physiologically compelling case for the post-translational action of SRP comes from studies of the chloroplast, in which protein synthesis and membrane targeting or integration can occur in physically distinct compartments of the cell\(^6\). Some integral membrane proteins are first synthesized in the cytosol and imported into the chloroplast through an N-terminal transit peptide, and the mature precursor only binds to the specialized chloroplast SRP (cpSRP) once delivered to the chloroplast stroma (BOX 2). The requirement for membrane-associated, cognate SRP receptors in these various pathways suggests that, even in post-translational mode, precursor delivery to the export membrane is an important feature of SRP function\(^{12,13}\).

**Elaborations on co-translational delivery**

Although the major components and mechanisms of SRP-dependent co-translational delivery are conserved between kingdoms, eukaryotic SRP has additional subunits that distinguishes it from that of prokaryotes. Furthermore, prokaryotes might also use a distinct co-translational route that is not found in the eukaryotic export pathway.

**Alternative pathways in prokaryotes.** In prokaryotes, the chaperone-dependent (normally post-translational) membrane delivery of some substrate domains might occur before protein synthesis is completed\(^6\). This process has been termed translation-linked delivery, and is conceptually distinct from true co-translational translocation, because only the precursor and not the translating ribosome is delivered to the export site by this effect\(^6\). However, the kinetics of this process and the interplay between this pathway and co- and post-translational delivery pathways are poorly understood.

Another facet of prokaryotic delivery is the composition of the export channel itself. Most bacterial proteins that are destined for export are delivered to the SecYEG export channel (see REF. 87 for a review) (BOX 1; FIGS 2,4). However, another protein, YidC, which is found in close association with the SecYEG complex, can also mediate the export and insertion of a subset of relatively small proteins into the inner membrane, and might also act independently of the SecYEG channel (FIG. 2). It is not clear precisely what features direct particular substrates to this ‘YidC-only’ pathway\(^7\). Only the small hydrophilic regions that are directly adjacent to transmembrane segments can be translocated by YidC. Whether delivery to YidC is also SRP dependent is unclear, and this might be substrate specific\(^8,9\). However, most data indicate that YidC-mediated insertion occurs co-translationally, which is consistent with a role for the SRP in the delivery step\(^9\) (FIG. 2). In eukaryotes, there is a mitochondrial inner membrane system that is analogous to the YidC-only prokaryotic pathway. A YidC homologue termed Oxal can bind to the ribosomal exit site, providing a molecular basis for a co-translational membrane insertion pathway\(^\#\#\). Although a corresponding ribosome docking site has not been identified in YidC, its early contact with short nascent polypeptides suggests that the ribosome and YidC can be in close proximity during membrane protein biogenesis\(^8\). At present, an equivalent Sec61-independent pathway has not been identified in the ER of eukaryotes, and the prevalence of this YidC-only route is unclear, even in prokaryotes\(^7\).

**Enhancing co-translational delivery in eukaryotes.**

Eukaryotic cells are larger and more complex than bacterial cells and mammalian SRP consists of an expanded RNA molecule, SRP54 and five additional protein subunits (SRP9, SRP14, SRP19, SRP68 and SRP72)\(^1\). Among these, SRP9 and SRP14 form the so-called Alu domain of eukaryotic SRP, which mediates the arrest of protein translation during the delivery step of protein export\(^1\). This domain is not found in bacteria\(^6\). It is suggested that the Alu domain interferes with ongoing protein synthesis, and the resulting slowing or arrest of protein elongation during co-translational delivery is thought to increase the efficiency of the targeting reaction\(^1,4,5\). The attenuation of translation prevents the synthesis of large regions of a polypeptide that could otherwise render the nascent polypeptide chain translocation incompetent or prone to aggregation, and compensates for the low number of SRP receptors in the ER membrane\(^1\).

In E. coli, SRP-mediated attenuation of translation does not occur\(^6\), but it seems that SRP-mediated delivery of proteins might occur by default, because it can be initiated while the nascent chain is still in the ribosomal exit tunnel, irrespective of the presence of a signal peptide\(^1\). Furthermore, the size and relative simplicity of prokaryotic cells probably means that the translating ribosome can remain in close proximity with the export membrane, preventing the need for a translational arrest function\(^5\).

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**Stroma**

The soluble region in the chloroplast that is functionally synonymous with bacterial cytosol.

**Alu domain**

A domain of the signal recognition particle (SRP) that comprises SRP9 and SRP14, together with helices three and four of the SRP RNA.
Plant cells contain a typical eukaryotic signal recognition particle (SRP) complex in the cytosol and a second SRP located in the chloroplast stroma (known as cpSRP)\(^4,33\). In addition to the cpSRP\(^54\) involved in conventional co-translational targeting\(^4,33\), the chloroplast stroma also contains a distinct population of cpSRP\(^54\) in complex with a unique protein, cpSRP\(^43\), which contains three chromodomains (CD1–3) and four ankyrin repeats (ANK1–4)\(^33\) (see the figure, part a). This cpSRP\(^54\)–cpSRP\(^43\) complex mediates the strictly post-translational delivery of genome-encoded precursor proteins to the thylakoid membrane. The biosynthesis of the light-harvesting chlorophyll a/b binding protein (LHCP) is considered to be a paradigm for this post-translational route\(^3,12\), and is an extremely abundant cargo for this specialized pathway\(^3,34,36\). Synthesis of LHCP begins in the cytosol (see the figure, part b), and the protein is post-translationally targeted to the chloroplast envelope (CE) through an amino-terminal transit peptide (TP). A distinct signal sequence in LHCP ensures that the potential for newly synthesized LHCP to bind to cytosolic SRP before import into the chloroplast is minimized, and the substrate is instead delivered to the chloroplast stroma through the TIC–TOC translocase machinery\(^30,102\). TP-cleaved, mature LHCP forms a delivery complex with the cpSRP through the binding of the polypeptide to both cpSRP\(^54\) and cpSRP\(^43\) subunits\(^84,112\). Intriguingly, the recent crystal structure of cpSRP\(^43\) (see the figure, part c)\(^41\) indicates that the overall shape and charge distribution of this protein resembles helix 8 of typical SRP RNAs (see the figure, part c)\(^41\) suggesting that cpSRP\(^43\) might substitute for the SRP RNA that is lacking from cpSRP. Hence, cpSRP\(^43\) acts as an adaptor, and, in combination with cpSRP\(^54\), binds to both the hydrophobic signal sequence of LHCP\(^112\) and a distinct cpSRP\(^43\)-specific SRP-binding motif that is also present in the polypeptide\(^84,113\). LHCP is delivered for export into the thylakoid membrane through the chloroplast FtsY homologue, cpFTSY (see the figure, part b)\(^31,103,36\). The thylakoid homologue of YidC, ALB3, then facilitates the membrane integration of the protein\(^84,103\).

In any case, the export of proteins that lack an export signal is most likely precluded by the proofreading capacity of the translocation channel, which prevents the translocation of inappropriately delivered precursors\(^84,102\). In eukaryotic cells, some mRNAs that encode secretory proteins also seem to be transported directly to the ER membrane in a process that is independent of either protein synthesis or any association with the ribosome. In this case, signals in the mRNA might be recognized directly to target them to specific subcellular locations. Such selective mRNA enrichment might begin with the transport of mRNAs that encode secretory proteins from the nucleus, a process that can be promoted by the nucleotide sequence that encodes the polypeptide-based export signal\(^103\). Several studies also point to the translation-independent subcellular localization of specific mRNAs that encode proteins other than those destined for secretion\(^104–108\). In this way, the initiation of translation for some eukaryotic proteins destined for export from the cell might occur at, or directly adjacent to, the membrane export machinery, and thereby minimize or even bypass the role of cytosolic delivery factors.

### Conclusions and perspectives

While the generic properties of the signals that direct proteins for export from the cytosol have been known for over 30 years, the subtle variations in export signal composition that function to direct precursors to specific export routes, such as HSP\(^40\) and HSP\(^70\) rather than ASNA\(^1\), or YidC rather than SecYEG, are either poorly defined or only just beginning to emerge\(^o\). We now have detailed mechanistic and structural insights into several different export pathways, most notably the co-translational SRP-dependent route, yet there are many gaps in our knowledge. For the post-translational export pathways, the true meaning of the frequently used term ‘translocation competent conformation’ is not well understood in molecular terms. Exactly how generic chaperones, such as HSP\(^40\) and HSP\(^70\), or specialized export factors, such as ASNA\(^1\), assist precursors in maintaining this state is also an open question. For the co-translational route, high-resolution structures of an export signal bound to a SRP\(^54\) family member are needed to better understand the molecular mechanisms behind this process.
Most studies of protein export have relied on either in vitro reconstitution or in vivo perturbation of individual routes. Thus, our current understanding of protein export at steady state and under normal growth conditions is limited — the development of non-invasive approaches to study protein export in real time is an obvious challenge for the future. Many studies have depended on a handful of model precursors to provide generic insights into protein delivery, and it is now clear that global approaches will be required to fully appreciate the complexities that underlie the protein export pathways outlined in this Review. However, a clear picture is now emerging that shows a substantial amount of overlap and redundancy between different protein delivery routes. The modularity of protein delivery and membrane translocation components allows the precise machineries and the pathway that are exploited by a substrate to be individually tailored to its needs. Such plasticity of protein export also provides an ideal basis for maintaining protein homeostasis during both chronic and acute environmental stresses. In this context, the recent discovery of an entirely novel post-translational protein delivery system in eukaryotes, the ASNA1 complex, provides a paradigm for understanding the molecular mechanisms by which stress can directly modulate the protein export process.
The structure of the SRP bound to a translating ribosome revealed the rearrangements that are necessary to drive co-translational delivery. 51


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**DATABASES**

UropoolKB: http://www.uropool.org

UniProtKB: http://www.uniprot.org

UniProtKB: http://www.uniprot.org

Cross’s homepage: http://www.uni-heidelberg.de/zentral/zhb

Stephen High’s homepage: http://www.lmu-mainz.ac.uk/peps/cgi/rmo?1d=6960+1

**FURTHER INFORMATION**

Benedict C. S. Cross’s homepage: http://www.lmu-mainz.ac.uk/peps/cgi/rmo?1d=6960+1

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