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Embracing the void—how much do we really know about targeting and translocation to the endoplasmic reticulum?

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In order for a protein to enter the secretory pathway, two crucial steps must occur: it first needs to be targeted to the cytosolic surface of the endoplasmic reticulum (ER), and then be translocated across the ER membrane. Although for many years studies of targeting focused on the signal recognition particle, recent findings reveal that several alternative targeting pathways exist, some still undescribed, and some only recently elucidated. In addition, many genes implicated in the translocation step have not been assigned a specific function. Here, we will focus on the open questions regarding ER targeting and translocation, and discuss how combining classical biochemistry with systematic approaches can promote our understanding of these essential cellular steps.

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The gaps between genomic overflow and shortage in functional characterization

In recent years, developments in the field of genomics have generated incomprehensible amounts of data. We now have the technical means to sequence every genome, to annotate the potential genes encoded in those sequences, and to predict functional motifs within the encoded proteins.

While complex organisms' genomes may be more challenging to decipher due to greater amounts of information, one would expect that the genome of a simple, unicellular, intensely studied model organism, such as the baker's yeast *Saccharomyces cerevisiae*, would be completely characterized. Despite that, here we are today, almost two decades after the sequencing of the entire yeast genome [1], still oblivious in regards to the biological functions of many of the 5853 yeast genes (www.yeastgenome.org). Though researchers continuously attribute functions to a growing number of genes, almost 25% of yeast genes are of unknown function. Of course, even for proteins for which a function has been assigned, much is left to uncover. Many of the unstudied proteins are localized to the various organelles (Figure 1), highlighting how little we know of the workings of even a simple eukaryotic cell.

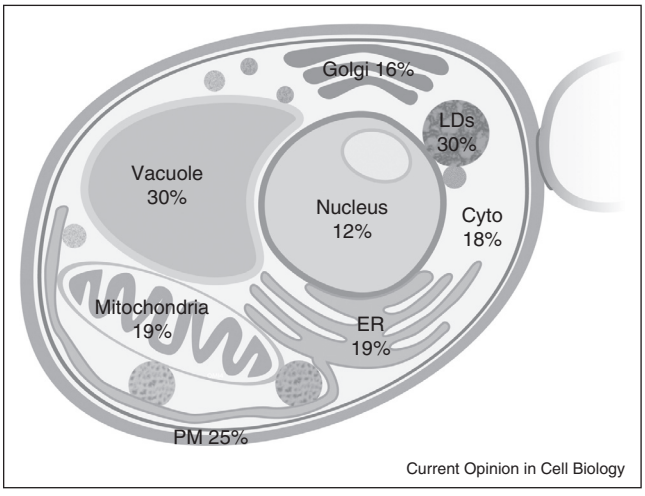
Modern-day's gap between the genomic overflow and functional characterization is a general issue in nearly every studied cell-biology question. In this review we will focus on one prime example, the entry of proteins into the secretory pathway. We will discuss the open questions in the field, and examine potential approaches to tackle them.

Entry into the secretory pathway—an example of an unresolved cellular process

Over 20% of any eukaryotic proteome is composed of secreted, membranal, and endomembrane organellar proteins. The correct distribution of these proteins relies on entry into the first station in the secretory pathway, the endoplasmic reticulum (ER), from which proteins are trafficked to their final destinations. Two things must occur in order for a protein to enter the ER: First, it has to be targeted in a regulated manner to a specific position on the ER membrane. Next, it needs to physically translocate through the ER membrane using dedicated machinery.

In 1971 Günter Blobel formulated the hypothesis that secretory proteins have intrinsic signals that govern their transport and localization in the cell (the signal hypothesis) and later received the Nobel Prize in medicine for proving it right. Soon after, in the 1980s, groundbreaking work in his lab discovered the existence of the signal recognition particle (SRP) and its receptor (SR), as the main pathway for ER targeting [2–6]. Not long after, the Sec61 translocon was shown to be the complex that enables translocation of proteins across the membrane [7,8]. Since their discovery, both the SRP-pathway and the Sec61 translocon have been extensively studied and their function and structure have been characterized to an atomic level [9,10••,11••,12–17]. Nevertheless, in spite of over thirty years of study, the field of ER targeting and translocation is far from being fully mapped. It is now clear that there are protein-groups that do not utilize the SRP-targeting pathway nor rely on the Sec61 translocon to insert into the membrane (Figure 2). Furthermore, along the years additional proteins were shown to modulate the translocation process, though the detailed function of many of them is still not fully understood. Thus, it appears that the full scope of mechanisms that enable efficient ER targeting and translocation has yet to be elucidated.

Figure 1



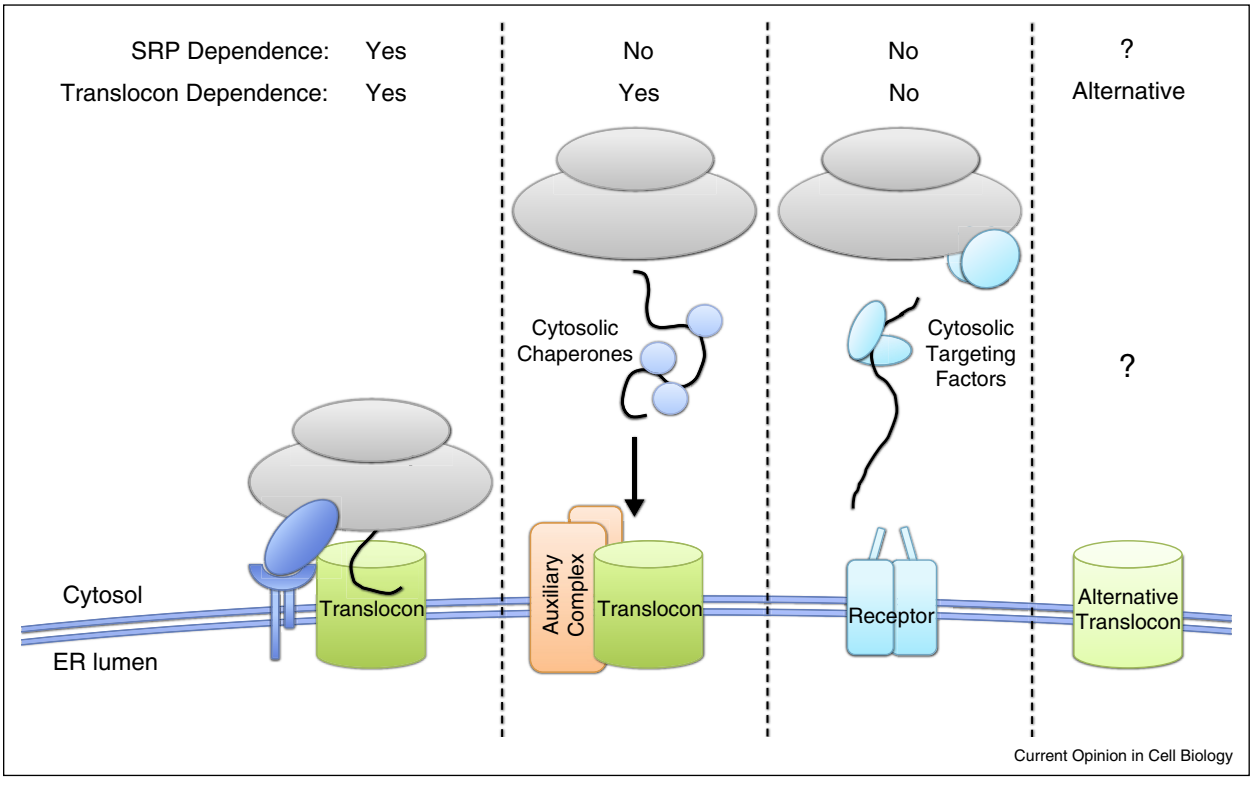
The function of many organellar proteins is yet unknown. Schematic representation of a yeast cell, showing the percentage of organellar proteins with unknown function. Localization data is based on C-terminally fluorescently tagged putative open reading frames, hence does not include proteins whose localization has not been demonstrated by this approach. Cyto, cytoplasm; ER, endoplasmic reticulum; LDs, lipid droplets; PM, plasma membrane.

ER-targeting pathways: recent discoveries and open questions

SRP mediated targeting is a very efficient process, though for both physical and regulatory reasons, it cannot support all ER-bound substrates. Consequently, all living cells have additional, SRP-independent, pathways for targeting a wide variety of substrates. It was shown that about a third of the *S. cerevisiae* secretome [18•] as well as a growing number of substrates in higher organisms [19•] are targeted to the ER in an SRP-independent manner.

One group of obligatory SRP-independent substrates are the tail-anchored (TA) proteins, which have a single transmembrane domain at their C-terminus, anchoring them to the cytosolic surface of organellar membranes and constituting their ER targeting sequence. Since the C-terminal tail leaves the ribosome exit tunnel only after translation termination, the SRP machinery cannot bind and target TA proteins cotranslationally. Markedly, TA proteins are not only SRP-independent, but are also translocon-independent [20••], since they do not fully translocate into the organellar lumen, but are anchored to the membrane. Only in the last few years have elegant biochemical approaches alongside new methods for functional genomics, uncovered the components of the targeting pathway: the TRC40 (Transmembrane Recognition

Figure 2



Several options exist for proteins to be targeted and translocated into the ER. Schematic representation of the major combinations between targeting and translocation machineries, demonstrated to be used by secretory proteins in eukaryotic cells.

Complex of 40 kDa) pathway in mammals [21,22] and the homologous yeast GET (Guided Entry of Tail-anchored proteins) pathway [23,24].

While the TRC40/GET pathway was originally discovered for targeting TA-proteins, recent studies have revealed a much larger substrate pool. The GET pathway was recently shown to facilitate the targeting of some, but not all, glycosylphosphatidylinositol (GPI)-anchored proteins in yeast [18•] by binding the hydrophobic GPI-anchoring domain in their C-terminus (pheno-mimicking the TA transmembrane domain). Additionally, the TRC40 targeting pathway was implicated in the targeting of very short secreted proteins [25••]. Because of their shortness, the translation of these proteins is completed while they are still accommodated in the ribosome's exit tunnel. As SRP binds the signal sequence (SS) of its substrate in a cotranslational manner, this group of proteins is not recognized by the SRP, and must rely on alternative targeting pathways [19•]. Since the targeting sequences of short secretory proteins are N-terminal and not highly hydrophobic, it is surprising that TRC40 recognizes them for ER targeting. Interestingly, in contrast to TA-proteins, both GPI-anchored and short secretory proteins are fully translocated through the translocon. Hence, this demonstrates that the TRC40/GET pathway is far more complex than previously appreciated and that regulation must exist to divert its substrates into a translocon dependent or independent insertion pathway.

Notably, many of the substrates that are targeted by the GET pathway are essential for viability, yet yeast deleted for the GET pathway, though sick, are still viable [18•,23]. Moreover, TRC40 inhibition doesn't seem to completely disrupt translocation of short secreted proteins [25••]. Taken together this suggests the presence of compensatory pathways. One possible compensation for TRC40 loss might be the newly described calmodulin-dependent pathway [26]. Calmodulin is a ubiquitous eukaryotic protein, which is involved in [Ca^{2+}]-dependent intracellular signaling [27]. It was shown to bind the SSs of short secreted proteins, keeping them in a translocation competent conformation and preventing their aggregation and degradation [26], as was suggested for other cytosolic chaperones [18•,28]. Interestingly, Sec61 has a calmodulin-binding motif in its cytosolic N-terminus [29], which suggests a possible role in ER-targeting. Calmodulin was also implicated as a negative regulator for TA-proteins insertion to the ER membrane [30], and as a chaperone for mitochondrial proteins [31]. It will be interesting to see if it acts as a general protein-binding factor for various organellar proteins, or if it has specific multiple functions depending on its substrate or the cellular Ca^{2+} concentration.

Despite the abovementioned recent discoveries, there is still uncertainty regarding the targeting of a large fraction of the secretome, composed of proteins with mildly hydrophobic SSs. Since the SRP binds its targeting substrate's SS with it's hydrophobic cleft [32], it is the extent of hydrophobicity of the substrate (amino acid composition and length) that determines binding to SRP [18•,33]. Many protein's SSs do not pass the binding threshold for SRP and thus would require an alternate, yet undiscovered, mechanism for targeting.

Markedly, even models of cellular processes that are thought to be well characterized, like the SRP targeting pathway, are continuously modified and expanded by new discoveries. Studies in bacteria suggest that despite the common belief, it is not SRP that targets the ribosomes to the membrane, but rather the SRP-receptor, which does so during its own translation [34•]. If also true in eukaryotes, then ribosomes with SRP would be waiting on the ER membrane and RNA molecules would have to be the ones targeted to the membrane rather than proteins. Hence, it is not surprising that mRNAs of subclasses of yeast secretory proteins seem to be targeted to the ER independently of the SRP via *cis*-acting sequences within their transcript [35]. Moreover, mammalian p180 has been shown to target the mRNA of a GPI-anchored protein to the ER membrane [36]. Hence, we have yet to unravel the regulatory network mediating efficient mRNA targeting to the ER membrane.

Unresolved issues in the ER-translocation process

While many of the ER targeting components are still unknown, most of the proteins which take part in translocation into the ER have been identified, yet their function is still little understood. One example of this is the basic unit of translocation, the Sec61 translocon. The translocon includes three proteins: Sec61α, the pore forming subunit, and two additional TA-proteins—Sec61β and Sec61γ [8]. Detailed experimentation has been performed on the structure and function of the conserved pore-forming α-subunit, both in prokaryotes and eukaryotes. This is not the case with the nonessential β-subunits and the essential γ-subunit. The two auxiliary subunits were suggested to act as stabilizers of the α-subunit [37,38], as the translocon gate [13,39,40], and as mediators between the translocon and the ribosome [41,42]. However, there is still no deep understanding regarding the functional connection between all three subunits, and how they act together to accomplish the translocation process.

Even Sec61α is proving to be more complex than a simple translocation channel. For example, luminal loop 7 of Sec61α was recently shown to effect only posttranslational translocation to the ER, with no effect on the SRP-dependent cotranslational translocation [43]. This finding is not surprising, since loop 7 of Sec61α was previously shown to bind the ER-luminal chaperone BiP

[44], which is an essential component of the SRP-independent translocon (see below). Furthermore, the Sec61 complex was shown to function as a monomer [12,45••], but is also observed as an oligomer [13,46]. There is a possibility that the translocon is dynamically regulated, and that several arrangement exist, each accounting for a different group of substrates.

In mammals, a few additional proteins were shown to effect translocation. One of them is TRAM (Translocating Chain Associated Membrane protein) which, along with the Sec61 complex, constitutes the minimal apparatus required for reconstitution of *in vitro* translocation [8]. In addition, other proteins such as TRAP, PAT-10, RAMP4 and p180 have been implicated in the translocation process (see Table 1 for a comprehensive list of targeting/translocation proteins and their suggested roles). Though some interesting pieces of evidence give clues to their potential roles, we are still far from understanding each of their specific functions, and how they interact together to accomplish well-coordinated translocation of various substrates.

The plot thickens when moving on to SRP-independent translocation. SRP-independent substrates utilize a specialized Sec61 translocon aided by the auxiliary SEC complex. The SEC complex is composed of the essential Sec62 and Sec63 proteins, both are conserved from yeast to humans [47–51]. In yeast, the complex also includes the nonessential Sec66 (also termed Sec71) and Sec72 proteins [52–55]. One of the crucial roles of the SEC complex is the recruitment of the essential ER-luminal chaperone Kar2/BiP (yeast/mammalian homologues), through a J-domain on the luminal loop of Sec63. Kar2/BiP provides the necessary driving force for energy-mediated posttranslational translocation of substrates through the translocon [51,56]. Notably, the division between SRP-dependent or independent translocon is not straightforward, as Sec63 and Kar2/BiP seem to take part in the early steps of SRP-dependent translocation as well [57,58]. Though intense studies were performed in the hope of elucidating the function of the SEC complex, the evidence we have today merely hints at the potential tasks of most of its components. Moreover, suggestions for additional proteins that might function in this complex still arise (Table 1). It is of great importance to define the role of each of these accessory proteins, in order to understand the biological solutions that are employed in the translocation of SRP-independent substrates as well as how specificity and regulation are achieved.

Another level of complexity arises from the fact that two types of ER translocons exist. In yeast the canonical translocon is built around the pore forming protein Sec61, and an alternate translocon is built around the non-essential Sec61 homologue, Ssh1 [59]. There is some debate as to whether the Ssh1 translocon takes part exclusively in co-translational translocation [41], or both in co and posttranslational translocation [60], and there is only one documented case in which a protein showed preferential targeting to the Ssh1 translocon [61]. In mammals the situation is rather similar with a canonical translocon built around Sec61α1 and an alternative translocon built around its homologue, Sec61α2 [20••], regarding which nothing is yet known. Many potential regulatory layers can arise from having two translocation systems in a cell: perhaps each is dedicated to a different substrate range, or directs clients to differing posttranslocational modifications? Maybe they are differentially regulated under various cellular conditions, or serve as backup machinery to help the cells under stress conditions when the flux of protein translocation is jamming the secretory pathway? The answers to these questions will expose basic biological principles that are of great importance.

Many bridges to cross

The biological processes that we chose to focus on in this review, targeting and translocation into the ER, exemplify how a process, studied for many years, can still remain obscure. Partly, this is an inherent feature of biology—as Einstein once said, 'As the circle of light increases, so does the circumference of darkness around it'. Nevertheless, an additional aspect that adds uncertainty may be that the first, classical works in the field, used model proteins to study translocation. Per definition—model proteins are the ones best behaved in systems aimed at studying the basal machinery. However, as research progresses it is now time to move beyond model proteins to study a large variety of substrates that will enable us to uncover the true complexity and beauty of biological pathways.

One way to move beyond the realm of model proteins is by defining the full substrate range of each pathway. This can be done by high content microscopic screens on fluorescently tagged substrates or mass spectrometric measurements of translocation efficiency in various genetic backgrounds. In addition to providing answers in the context of pathway specificity, such studies are also essential for revealing the relative prevalence of each pathway under different physiological conditions. Moreover, once an entire substrate range has been uncovered, bioinformatic analyses can bring out common features responsible for the interaction.

An additional challenge in revealing the alternative targeting pathways for the SRP-independent substrates is functional redundancy. It seems that proteins that are involved in targeting of SRP-independent substrates do not act exclusively, and upon the loss of one targeting pathway the cell has backup networks. A graceful way to tackle this problem, using the immense genomic data that we have today, is the systematic analysis of genetic interactions [105]. This approach can use any quantitative phenotype to measure the difference between the effect

Table 1

Overview of the genes known to take part in chaperoning, targeting and translocation to the ER Suggested functions of mammalian and yeast genes in the chaperoning, targeting and translocation to the endoplasmic reticulum (ER). Additional functions that are not related to targeting or translocation are not listed. The involvement of each gene in SRP-dependent targeting or the translocon-dependent translocation is indicated on the left column. *In cases where cellular-roles are conserved, functions based on studies in bacteria/archaea are also mentioned. SRP, signal recognition particle; SR, SRP-receptor; SS, signal sequence; RNC, ribosome nascent chain complex; TA, tail-anchored; TM, trans membrane; GEF, GTP exchange factor; GPI, glycosylphosphatidylinositol; ER, endoplasmic reticulum

	Mammals	Yeast	Suggested function
Targeting/chaperoning			
Signal recognition particle (SRP) pathway			
◆○	SRP	SRP	Binding translating ribosomes from which hydrophobic SSs are emerging (mammals [2,3,9]; yeast [14]) Arresting translation elongation (mammals [4]; yeast [15]) Targeting RNCs to the SR (mammals [9]; yeast [16])
◆○	SRα & SRβ	Srp101 & Srp102	Functioning as the SRP receptor for targeting SRP-RNCs; releasing translation arrest (mammals [5,6]; yeast [16]) Transferring SRP-RNCs to the translocon for cotranslational translocation (mammals [17]; yeast [41]) Proofreading substrate specificity via kinetics of SRP-SR complex assembly and GTP hydrolysis (bacteria [62]) Targeting ribosomes to the ER membrane during SR mRNA translation (bacteria [34•])
Cytosolic chaperones			
❖ ○	Hsc70s	Ssa1	Maintaining posttranslational precursors in a translocation-competent conformation (mammals [63]; yeast [28]) Facilitating membrane integration of a subset of TA proteins (mammals [64])
❖ ○	Hsp40s	Ydj1; Apj1; Jjj3	Recruiting Hsp70s for substrate-specific chaperoning of SRP-independent proteins (yeast [18•]) Facilitating membrane integration of a subset of TA proteins (mammals [64])
❖ ○	CaM	Cmd1	SS-dependent chaperoning for small secretory proteins (mammals [26]) Limiting calcium leakage from the ER through interaction with Sec61 (mammals [29]) Chaperoning of mitochondrial proteins (kinetoplastids [31])
TRC40/GET pathway			
❖ ○ ⊙	SGTA	Sgt2	Capturing newly synthesized TA proteins (yeast [65•])
❖ ○ ⊙	Bag6		Capturing newly synthesized TA proteins (mammals [66,67])
❖ ○ ⊙	TRC35 & Ubl4a	Get4 & Get5	Transfering TA proteins from Bag6/Sgt2 to TRC40/Get3 (mammals [66,67]; yeast [24,65•,68])
❖ ○ ⊙	TRC40	Get3	Posttranslational ER-targeting of TA proteins (mammals [21,22]; yeast [23]) Posttranslational ER-targeting of short secreted proteins (mammals [25••]) ER-targeting of GPI-anchored proteins (yeast [18•]) Preventing substrate aggregation when ER targeting is blocked (yeast [69])
❖ ○ ⊙	WRB & CAML	Get1 & Get2	Functioning as the Trc40/GET3 receptor, mediating insertion of TA protein (mammals [70,71]; yeast [23,65•,72,73])
Translocons			
Canonical translocon			
◆❖ ○	Sec61α1	Sec61	Pore forming (mammals [12,74]; yeast [12,75]; archaea [13]; bacteria [10••]) Ribosome docking (mammals [12,76]; yeast [12,77]) Mediating both cotranslational and posttranslational translocation (mammals [20••]; yeast [55,78]) Recognizing SSs (mammals [39]; yeast [79])
◆❖ ○	Sec61β	Sbh1	Recruiting the signal peptidase complex (mammals [38]) Binding ribosomes via its cytoplasmic domain (mammals [42]) Signaling translocon vacancy to the SRP–SR–RNC complex via its TM domain (yeast [41]) Acting as a GEF for SRβ (yeast [80])
◆❖ ○	Sec61γ	Sss1	Translocon-gating through replacement of the SS in unoccupied translocons (yeast [39]) Coordinating translocon opening as a clamp (yeast [40]; archaea [13]) Supporting translocon architectural integrity and dynamic function (yeast [37])

Table 1 (*Continued*)

	Mammals	Yeast	Suggested function
Alternative translocon			
◆❖ O	Sec61α2	Ssh1	Pore forming for cotranslational translocation (yeast [41,59]) Pore forming for both cotranslational and posttranslational translocation (yeast [60]) Translocating Sec71 (yeast [61])
◆❖ O	Sec61β	Sbh2	Signaling translocon vacancy to the SRP–SR–RNC complex via its TM domain (yeast [41]) Mediating preferential targeting of Sec71 to the Ssh1 translocation pore (yeast [61])
◆❖ O	Sec61γ	Sss1	Shared subunit with canonical translocon (as above)
Auxiliary translocation factors			
◆O	TRAM		Inserting SSs to the translocon pore (mammals [81]) Integrating TM domains into the membrane (mammals [82]) Regulating translocational-pausing and nascent chain exposure to the cytosol (mammals [83]) Regulating membrane protein biogenesis at the translocon (mammals [84,85])
◆O	TRAP complex		Initiating substrate-specific translocation via the SS (mammals [86]) Controlling topogenesis of membrane proteins (mammals [87])
◆ O	RAMP4	Ysy6	Regulating protein modification by translocational-pausing (mammals [88]) Stabilizing newly synthesized membrane proteins under ER stress (mammals [89]) Maintaining ER-homeostasis and translocation efficiency (mammals [90])
◆ O	PAT-10		Coordinating membrane integration of polytopic membrane proteins (mammals [82,91])
◆❖ O	p180		Tethering ribosomes on the ER membrane (mammals [92]) Facilitating ER translocation of large extracellular matrix proteins (mammals [93]) Ribosome-independent anchoring of mRNA to the ER (mammals [36]) No stimulatory role in translocation (mammals [76])
O	ERj1		Coordinating BiP-mediated translocation by signaling between ribosomes and BiP (mammals [94,95])
SEC complex			
❖ O	Sec62	Sec62	Docking SSs during substrate targeting to the translocation channel (yeast [39,79]) Mediating SS-dependent posttranslational translocation of short secreted proteins (mammals [96•]) Mediating SS-independent translocation of short secreted proteins (mammals [97]) Regulating orientation of moderately hydrophobic signal-anchors during posttranslational translocation (yeast [98]) Binding the ribosome exit tunnel (mammals [99]) Sec63 binding and SEC complex stabilization (mammals [99]; yeast [100])
◆❖ O	Sec63	Sec63	Inserting specific substrates of both cotranslational and posttranslational translocation into the translocon (mammals [20••]; yeast [57]) Initiating cotranslational translocation (yeast [58]) Mediating SS-independent translocation of short secreted proteins (mammals [97]) Mediating Kar2/ATP-dependent release and pore-insertion of posttranslational substrates via its luminal J-domain (yeast [51,56]) Assembling and stabilizing SEC complexes (yeast [58,100]) Regulating Sec62 recruitment to the SEC complex (mammals [99]; yeast [100,101])
❖ O		Sec66/71	Docking SSs during targeting of nascent chains to the translocation channel (yeast [39,52,79]) Transporting a subset of posttranslational precursors to the translocon (yeast [101]) Facilitating SEC complex assembly/stability (yeast [52])
❖ O		Sec72	Docking SSs during targeting of nascent chains to the translocation channel (yeast [102]) Transporting a subset of posttranslational precursors to the translocon (yeast [101,102]) Recognizing cytosolic Hsp70s (yeast [103]) Retaining the interaction between Sec63 and Sec71 (yeast [102])
❖ O		Hph1 & Hph2	Promoting posttranslational translocation of V-ATPase biogenesis factors (yeast [104])

◆, SRP-dependent; ❖, SRP-independent; O, translocon-dependent; ⊙, translocon-independent.

of a single mutation and that of a double mutation. Large-scale genome-wide genetic analysis provides an unbiased approach for revealing new unknown interactions. Furthermore, based on each gene's interaction map, they can be clustered into groups with similar profiles—which indicate a common function. In the context of ER targeting, these systematic approaches in yeast have already given rise to the discovery of the GET pathway [24,105] and the network of proteins that are involved in the chaperoning of SRP-independent substrates [18•].

The lack of detailed functional information regarding the proteins in the translocation apparatus highlights the need for complementary biochemical methodologies alongside the abovementioned functional-genomics approaches. New cutting edge *in situ* structural analyses are starting to reveal the spatial organization of the native mammalian translocon and its auxiliary components [106•], and it will be very appealing to apply these methods for more hypothesis-driven functional studies.

In modern days, it is easy to find yourself standing in front of the paralyzing genomic pool, overwhelmed by data and options, not knowing how or where to start answering questions. Combining systematic approaches and data with in depth biochemical reconstitution should enable us to uncover the remaining pathways, shed light on some of the mysteries of targeting and translocation and gain a fuller understanding of the cellular processes that enable life.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M *et al.*: **Life with 6000 genes**. *Science* 1996, **274**:563-567.

2. Walter P, Ibrahimi I, Blobel G: **Translocation of proteins across the endoplasmic reticulum I. Signal recognition protein (SRP) binds to in-vitro-assembled polysomes synthesizing secretory protein**. *J Cell Biol* 1981, **91**:545-550.

3. Walter P, Blobel G: **Translocation of proteins across the endoplasmic reticulum II. Signal recognition protein (SRP) mediates the selective binding to microsomal membranes of in-vitro-assembled polysomes synthesizing secretory protein**. *J Cell Biol* 1981, **91**:551-556.

4. Walter P, Blobel G: **Translocation of proteins across the endoplasmic reticulum III. Signal recognition protein (SRP) causes signal sequence-dependent and site-specific arrest of chain elongation that is released by microsomal membranes**. *J Cell Biol* 1981, **91**:557-561.

5. Gilmore R, Blobel G, Walter P: **Protein translocation across the endoplasmic reticulum I. Detection in the microsomal membrane of a receptor for the signal recognition particle**. *J Cell Biol* 1982, **95**:463-469.

6. Gilmore R, Walter P, Blobel G: **Protein translocation across the endoplasmic reticulum II. Isolation and characterization of the signal recognition particle receptor**. *J Cell Biol* 1982, **95**:470-477.

7. Deshaies RJ, Schekman R: **A yeast mutant defective at an early stage in import of secretory protein precursors into the endoplasmic reticulum**. *J Cell Biol* 1987, **105**:633-645.

8. Görlich D, Rapoport TA: **Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane**. *Cell* 1993, **75**:615-630.

9. Halic M, Blau M, Becker T, Mielke T, Pool MR, Wild K, Sinning I, Beckmann R: **Following the signal sequence from ribosomal tunnel exit to signal recognition particle**. *Nature* 2006, **444**:507-511.

10. •• Park E, Ménétret JF, Gumbart JC, Ludtke SJ, Li W, Whynot A, Rapoport TA, Akey CW: **Structure of the SecY channel during initiation of protein translocation**. *Nature* 2014, **506**:102-106.
This is the most recent structural work studying the SecY translocon complex. The structures of active and inactive ribosome-translocon complexes were resolved, revealing new insights regarding the role of the nascent chain in the channel opening.

11. •• Gogala M, Becker T, Beatrix B, Armache JP, Barrio-Garcia C, Berninghausen O, Beckmann R: **Structures of the Sec61 complex engaged in nascent peptide translocation or membrane insertion**. *Nature* 2014, **506**:107-110.
This work presents a structural model of the mamalian Sec61 complex engaged in translocation, confirming its basic activities as a protein-conducting channel.

12. Becker T, Bhushan S, Jarasch A, Armache JP, Funes S, Jossinet F, Gumbart J, Mielke T, Berninghausen O, Schulten K *et al.*: **Structure of monomeric yeast and mammalian Sec61 complexes interacting with the translating ribosome**. *Science* 2009, **326**:1369-1373.

13. Van den Berg B, Clemons WM, Collinson I, Modis Y, Hartmann E, Harrison SC, Rapoport TA: **X-ray structure of a protein-conducting channel**. *Nature* 2004, **427**:36-44.

14. Ogg SC, Walter P: **SRP samples nascent chains for the presence of signal sequences by interacting with ribosomes at a discrete step during translation elongation**. *Cell* 1995, **81**:1075-1084.

15. Mason N, Ciufo LF, Brown JD: **Elongation arrest is a physiologically important function of signal recognition particle**. *EMBO J* 2000, **19**:4164-4174.

16. Hann BC, Walter P: **The signal recognition particle in *S. cerevisiae***. *Cell* 1991, **67**:131-144.

17. Mandon EC, Jiang Y, Gilmore R: **Dual recognition of the ribosome and the signal recognition particle by the SRP receptor during protein targeting to the endoplasmic reticulum**. *J Cell Biol* 2003, **162**:575-585.

18. • Ast T, Cohen G, Schuldiner M: **A network of cytosolic factors targets SRP-independent proteins to the endoplasmic reticulum**. *Cell* 2013, **152**:1134-1145.
The involvement of chaperones in posttranslational targeting to the endoplasmic reticulum has been known for a long time, though only a few proteins were shown to be directly involved in this process. Here, a systematic screen reveled the specific involvement of several chaperones in this process. In addition, a new group of SRP-independent substrates was shown to use the GET pathway (previously described as a targeting pathway for tail-anchored proteins).

19. • Ast T, Schuldiner M: **All roads lead to Rome (but some may be harder to travel): SRP-independent translocation into the endoplasmic reticulum**. *Crit Rev Biochem Mol Biol* 2013, **48**:273-288.
Many proteins destined to the endoplasmic reticulum cannot facilitate the SRP-pathway. This is a comprehensive review discussing the reasons for a substrate's SRP-independence, as well as the chaperoning, targeting and insertion mechanisms of alternative pathways.

20. •• Lang S, Benedix J, Fedeles SV, Schorr S, Schirra C, Schäuble N, Jalal C, Greiner M, Hassdenteufel S, Tatzelt J *et al.*: **Different effects of Sec61α, Sec62 and Sec63 depletion on transport of polypeptides into the endoplasmic reticulum of mammalian cells**. *J Cell Sci* 2012, **125**:1958-1969.

This work maps the effect of SEC protein depletion in mammalian cells, and elegantly showed the involvement of each protein in the translocation of cotranslational and posttranslational substrates.

21. Stefanovic S, Hegde RS: **Identification of a targeting factor for posttranslational membrane protein insertion into the ER**. *Cell* 2007, **128**:1147-1159.

22. Favaloro V, Vilardi F, Schlecht R, Mayer MP, Dobberstein B: **Asna1/TRC40-mediated membrane insertion of tail-anchored proteins**. *J Cell Sci* 2010, **123**:1522-1530.

23. Schuldiner M, Metz J, Schmid V, Denic V, Rakwalska M, Schmitt HD, Schwappach B, Weissman JS: **The GET complex mediates insertion of tail-anchored proteins into the ER membrane**. *Cell* 2008, **134**:634-645.

24. Jonikas MC, Collins SR, Denic V, Oh E, Quan EM, Schmid V, Weibezahn J, Schwappach B, Walter P, Weissman JS *et al.*: **Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum**. *Science* 2009, **323**:1693-1697.

25. •• Johnson N, Vilardi F, Lang S, Leznicki P, Zimmermann R, High S: **TRC40 can deliver short secretory proteins to the Sec61 translocon**. *J Cell Sci* 2012, **125**:4414.
The TRC40 pathway was previously indicated as the targeting machinery of tail-anchored proteins to the endoplasmic reticulum. In this work, a new group of substrates, short secretory proteins, was shown to use the TRC40 pathway, suggesting we still have much more to find out regarding this pathway and its regulation.

26. Shao S, Hegde RS: **A calmodulin-dependent translocation pathway for small secretory proteins**. *Cell* 2011, **147**:1576-1588.

27. Chin D, Means AR: **Calmodulin: a prototypical calcium sensor**. *Trends Cell Biol* 2000, **10**:322-328.

28. Ngosuwan J, Wang NM, Fung KL, Chirico WJ: **Roles of cytosolic Hsp70 and Hsp40 molecular chaperones in post-translational translocation of presecretory proteins into the endoplasmic reticulum**. *J Biol Chem* 2003, **278**:7034-7042.

29. Erdmann F, Schäuble N, Lang S, Jung M, Honigmann A, Ahmad M, Dudek J, Benedix J, Harsman A, Kopp A *et al.*: **Interaction of calmodulin with Sec61α limits Ca2+ leakage from the endoplasmic reticulum**. *EMBO J* 2011, **30**:17-31.

30. Hassdenteufel S, Schäuble N, Cassella P, Leznicki P, Müller A, High S, Jung M, Zimmermann R: **Ca2+-calmodulin inhibits tail-anchored protein insertion into the mammalian endoplasmic reticulum membrane**. *FEBS Lett* 2011, **585**:3485-3490.

31. Aich A, Shaha C: **Novel role of calmodulin in regulating protein transport to mitochondria in a unicellular eukaryote**. *Mol Cell Biol* 2013, **33**:4579-4593.

32. Keenan RJ, Freymann DM, Walter P, Stroud RM: **Crystal structure of the signal sequence binding subunit of the signal recognition particle**. *Cell* 1998, **94**:181-191.

33. Ng DT, Brown JD, Walter P: **Signal sequences specify the targeting route to the endoplasmic reticulum membrane**. *J Cell Biol* 1996, **134**:269-278.

34. • Bibi E: **Is there a twist in the *Escherichia coli* signal recognition particle pathway?** *Trends Biochem Sci* 2012, **37**:1-6.
This paper presents an alternative hypothesis to the canonical targeting pathway, and suggests that the in vivo targeting of ribosomes to the membrane is mediated by the SRP-receptor rather than by the SRP itself. It proposes thought provoking ideas regarding the biogenesis of bacterial membrane proteins, which are possibly relevant to other organisms as well.

35. Kraut-Cohen J, Afanasieva E, Haim-Vilmovsky L, Slobodin B, Yosef I, Bibi E, Gerst JE: **Translation- and SRP-independent mRNA targeting to the endoplasmic reticulum in the yeast *Saccharomyces cerevisiae***. *Mol Biol Cell* 2013, **24**:3069-3084.

36. Cui XA, Zhang Y, Hong SJ, Palazzo AF: **Identification of a region within the placental alkaline phosphatase mRNA that mediates p180-dependent targeting to the endoplasmic reticulum**. *J Biol Chem* 2013, **288**:29633-29641.

37. Falcone D, Henderson MP, Nieuwland H, Coughlan CM, Brodsky JL, Andrews DW: **Stability and function of the Sec61 translocation complex depends on the Sss1p tail-anchor sequence**. *Biochem J* 2011, **436**:291-303.

38. Kalies KU, Rapoport TA, Hartmann E: **The beta subunit of the Sec61 complex facilitates cotranslational protein transport and interacts with the signal peptidase during translocation**. *J Cell Biol* 1998, **141**:887-894.

39. Plath K, Mothes W, Wilkinson BM, Stirling CJ, Rapoport TA: **Signal sequence recognition in posttranslational protein transport across the yeast ER membrane**. *Cell* 1998, **94**:795-807.

40. Wilkinson BM, Brownsword JK, Mousley CJ, Stirling CJ: **Sss1p is required to complete protein translocon activation**. *J Biol Chem* 2010, **285**:32671-32677.

41. Jiang Y, Cheng Z, Mandon EC, Gilmore R: **An interaction between the SRP receptor and the translocon is critical during cotranslational protein translocation**. *J Cell Biol* 2008, **180**:1149-1161.

42. Levy R, Wiedmann M, Kreibich G: **In vitro binding of ribosomes to the beta subunit of the Sec61p protein translocation complex**. *J Biol Chem* 2001, **276**:2340-2346.

43. Tretter T, Pereira FP, Ulucan O, Helms V, Allan S, Kalies KU, Römisch K: **ERAD and protein import defects in a sec61 mutant lacking ER-lumenal loop 7**. *BMC Cell Biol* 2013, **14**:56.

44. Schäuble N, Lang S, Jung M, Cappel S, Schorr S, Ulucan Ö, Linxweiler J, Dudek J, Blum R, Helms V *et al.*: **BiP-mediated closing of the Sec61 channel limits Ca2+ leakage from the ER**. *EMBO J* 2012, **31**:3282-3296.

45. •• Harada Y, Li H, Wall JS, Li H, Lennarz WJ: **Structural studies and the assembly of the heptameric post-translational translocon complex**. *J Biol Chem* 2011, **286**:2956-2965.
The SEC complex, associated with the endoplasmic reticulum translocon, is known to facilitate the posttranslational translocation of many SRP-independent substrates. Here, a combination of cryo-electron microscopy and dissociation patterns, gave rise to a model of the SEC complex architecture, which provides a big step towards the understanding of this enigmatic complex's functions.

46. Ménétret JF, Hegde RS, Heinrich SU, Chandramouli P, Ludtke SJ, Rapoport Ta, Akey CW: **Architecture of the ribosome-channel complex derived from native membranes**. *J Mol Biol* 2005, **348**:445-457.

47. Meyer HA, Grau H, Kraft R, Kostka S, Prehn S, Kalies KU, Hartmann E: **Mammalian Sec61 is associated with Sec62 and Sec63**. *J Biol Chem* 2000, **275**:14550-14557.

48. Deshaies RJ, Schekman R: **SEC62 encodes a putative membrane protein required for protein translocation into the yeast endoplasmic reticulum**. *J Cell Biol* 1989, **109**:2653-2664.

49. Rothblatt JA, Deshaies RJ, Sanders SL, Daum G, Schekman R: **Multiple genes are required for proper insertion of secretory proteins into the endoplasmic reticulum in yeast**. *J Cell Biol* 1989, **109**:2641-2652.

50. Kurihara T, Silver P: **Suppression of a sec63 mutation identifies a novel component of the yeast endoplasmic reticulum translocation apparatus**. *Mol Biol Cell* 1993, **4**:919-930.

51. Brodsky JL, Schekman R: **A Sec63p-BiP complex from yeast is required for protein translocation in a reconstituted proteoliposome**. *J Cell Biol* 1993, **123**:1355-1363.

52. Feldheim D, Yoshimura K, Admon A, Schekman R: **Structural and functional characterization of Sec66p, a new subunit of the polypeptide translocation apparatus in the yeast endoplasmic reticulum**. *Mol Biol Cell* 1993, **4**:931-939.

53. Green N, Fang H, Walter P: **Mutants in three novel complementation groups inhibit membrane protein insertion into and soluble protein translocation across the endoplasmic reticulum membrane of *Saccharomyces cerevisiae***. *J Cell Biol* 1992, **116**:597-604.

54. Deshaies RJ, Sanders SL, Feldheim DA, Schekman R: **Assembly of yeast Sec proteins involved in translocation into the endoplasmic reticulum into a membrane-bound multisubunit complex**. *Nature* 1991, **349**:806-808.

55. Panzner S, Dreier L, Hartmann E, Kostka S, Rapoport TA: **Posttranslational protein transport in yeast reconstituted with a purified complex of Sec proteins and Kar2p**. *Cell* 1995, **81**:561-570.

56. Matlack KE, Plath K, Misselwitz B, Rapoport TA: **Protein transport by purified yeast Sec complex and Kar2p without membranes**. *Science* 1997, **277**:938-941.

57. Brodsky JL, Goeckeler J, Schekman R: **BiP and Sec63p are required for both co- and posttranslational protein translocation into the yeast endoplasmic reticulum**. *Proc Natl Acad Sci USA* 1995, **92**:9643-9646.

58. Young BP, Craven RA, Reid PJ, Willer M, Stirling CJ: **Sec63p and Kar2p are required for the translocation of SRP-dependent precursors into the yeast endoplasmic reticulum in vivo**. *EMBO J* 2001, **20**:262-271.

59. Finke K, Plath K, Panzner S, Prehn S, Rapoport TA, Hartmann E, Sommer T: **A second trimeric complex containing homologs of the Sec61p complex functions in protein transport across the ER membrane of *S. cerevisiae***. *EMBO J* 1996, **15**:1482-1494.

60. Wilkinson BM, Tyson JR, Stirling CJ: **Ssh1p determines the translocation and dislocation capacities of the yeast endoplasmic reticulum**. *Dev Cell* 2001, **1**:401-409.

61. Spiller MP, Stirling CJ: **Preferential targeting of a signal recognition particle-dependent precursor to the Ssh1p translocon in yeast**. *J Biol Chem* 2011, **286**:21953-21960.

62. Zhang X, Rashid R, Wang K, Shan S: **Sequential checkpoints govern substrate selection during cotranslational protein targeting**. *Science* 2010, **328**:757-760.

63. Zimmermann R, Sagstetter M, Lewis MJ, Pelham HR: **Seventy-kilodalton heat shock proteins and an additional component from reticulocyte lysate stimulate import of M13 procoat protein into microsomes**. *EMBO J* 1988, **7**:2875-2880.

64. Rabu C, Wipf P, Brodsky JL, High S: **A precursor-specific role for Hsp40/Hsc70 during tail-anchored protein integration at the endoplasmic reticulum**. *J Biol Chem* 2008, **283**: 27504-27513.

65. • Wang F, Whynot A, Tung M, Denic V: **The mechanism of tail-anchored protein insertion into the ER membrane**. *Mol Cell* 2011, **43**:738-750.
In this study biochemical reconstitution was used to reveal the cyclic activity of the GET pathway. The study shows that Get3 is first tethered to the endoplasmic reticulum by interacting with Get2, and then releases its substrate through interactions with Get1, thus becoming available for another round of targeting. By defining the sequence of events, a better understanding of this conserved pathway was achieved.

66. Mariappan M, Li X, Stefanovic S, Sharma A, Mateja A, Keenan RJ, Hegde RS: **A ribosome-associating factor chaperones tail-anchored membrane proteins**. *Nature* 2010, **466**:1120-1124.

67. Leznicki P, Clancy A, Schwappach B, High S: **Bat3 promotes the membrane integration of tail-anchored proteins**. *J Cell Sci* 2010, **123**:2170-2178.

68. Kohl C, Tessarz P, von der Malsburg K, Zahn R, Bukau B, Mogk A: **Cooperative and independent activities of Sgt2 and Get5 in the targeting of tail-anchored proteins**. *Biol Chem* 2011, **392**:601-608.

69. Powis K, Schrul B, Tienson H, Gostimskaya I, Breker M, High S, Schuldiner M, Jakob U, Schwappach B: **Get3 is a holdase chaperone and moves to deposition sites for aggregated proteins when membrane targeting is blocked**. *J Cell Sci* 2013, **126**:473-483.

70. Yamamoto Y, Sakisaka T: **Molecular machinery for insertion of tail-anchored membrane proteins into the endoplasmic reticulum membrane in mammalian cells**. *Mol Cell* 2012, **48**:387-397.

71. Vilardi F, Stephan M, Clancy A, Janshoff A, Schwappach B: **WRB and CAML are necessary and sufficient to mediate tail-anchored protein targeting to the ER membrane**. *PLoS ONE* 2014, **9**:e85033.

72. Mariappan M, Mateja A, Dobosz M, Bove E, Hegde RS, Keenan RJ: **The mechanism of membrane-associated steps in tail-anchored protein insertion**. *Nature* 2011, **477**:61-66.

73. Stefer S, Reitz S, Wang F, Wild K, Pang Y, Schwarz D, Bomke J, Hein C, Löhr F, Bernhard F *et al.*: **Structural basis for tail-anchored membrane protein biogenesis by the Get3-receptor complex**. *Science* 2011, **333**:758-762.

74. Wirth A, Jung M, Bies C, Frien M, Tyedmers J, Zimmermann R, Wagner R: **The Sec61p complex is a dynamic precursor activated channel**. *Mol Cell* 2003, **12**:261-268.

75. Beckmann R, Spahn CM, Eswar N, Helmers J, Penczek PA, Sali a, Frank J, Blobel G: **Architecture of the protein-conducting channel associated with the translating 80S ribosome**. *Cell* 2001, **107**:361-372.

76. Kalies KU, Görlich D, Rapoport TA: **Binding of ribosomes to the rough endoplasmic reticulum mediated by the Sec61p-complex**. *J Cell Biol* 1994, **126**:925-934.

77. Prinz a, Hartmann E, Kalies KU: **Sec61p is the main ribosome receptor in the endoplasmic reticulum of *Saccharomyces cerevisiae***. *Biol Chem* 2000, **381**:1025-1029.

78. Stirling CJ, Rothblatt J, Hosobuchi M, Deshaies R, Schekman R: **Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum**. *Mol Biol Cell* 1992, **3**:129-142.

79. Plath K, Wilkinson BM, Stirling CJ, Rapoport TA: **Interactions between Sec complex and prepro-alpha-factor during posttranslational protein transport into the endoplasmic reticulum**. *Mol Biol Cell* 2004, **15**:1-10.

80. Helmers J, Schmidt D, Glavy JS, Blobel G, Schwartz T: **The beta-subunit of the protein-conducting channel of the endoplasmic reticulum functions as the guanine nucleotide exchange factor for the beta-subunit of the signal recognition particle receptor**. *J Biol Chem* 2003, **278**:23686-23690.

81. Voigt S, Jungnickel B, Hartmann E, Rapoport TA: **Signal sequence-dependent function of the TRAM protein during early phases of protein transport across the endoplasmic reticulum membrane**. *J Cell Biol* 1996, **134**:25-35.

82. Meacock SL, Lecomte FJL, Crawshaw SG, High S: **Different transmembrane domains associate with distinct endoplasmic reticulum components during membrane integration of a polytopic protein**. *Mol Biol Cell* 2002, **13**:4114-4129.

83. Hegde RS, Voigt S, Rapoport TA, Lingappa VR: **TRAM regulates the exposure of nascent secretory proteins to the cytosol during translocation into the endoplasmic reticulum**. *Cell* 1998, **92**:621-631.

84. Cross BCS, High S: **Dissecting the physiological role of selective transmembrane-segment retention at the ER translocon**. *J Cell Sci* 2009, **122**:1768-1777.

85. Tamborero S, Vilar M, Martínez-Gil L, Johnson AE, Mingarro I: **Membrane insertion and topology of the translocating chain-associating membrane protein (TRAM)**. *J Mol Biol* 2011, **406**:571-582.

86. Fons RD, Bogert BA, Hegde RS: **Substrate-specific function of the translocon-associated protein complex during translocation across the ER membrane**. *J Cell Biol* 2003, **160**:529-539.

87. Sommer N, Junne T, Kalies KU, Spiess M, Hartmann E: **TRAP assists membrane protein topogenesis at the mammalian ER membrane**. *Biochim Biophys Acta* 2013, **1833**:3104-3111.

88. Schröder K, Martoglio B, Hofmann M, Hölscher C, Hartmann E, Prehn S, Rapoport TA, Dobberstein B: **Control of glycosylation of MHC class II-associated invariant chain by translocon-associated RAMP4**. *EMBO J* 1999, **18**:4804-4815.

89. Yamaguchi A, Hori O, Stern DM, Hartmann E, Ogawa S, Tohyama M: **Stress-associated endoplasmic reticulum protein 1 (SERP1)/ribosome-associated membrane protein 4 (RAMP4) stabilizes membrane proteins during stress and facilitates subsequent glycosylation**. *J Cell Biol* 1999, **147**: 1195-1204.

90. Hori O, Miyazaki M, Tamatani T, Ozawa K, Takano K, Okabe M, Ikawa M, Hartmann E, Mai P, Stern DM *et al.*: **Deletion of SERP1/RAMP4, a component of the endoplasmic reticulum (ER) translocation sites, leads to ER stress**. *Mol Cell Biol* 2006, **26**:4257-4267.

91. Ismail N, Crawshaw SG, Cross BCS, Haagsma AC, High S: **Specific transmembrane segments are selectively delayed at the ER translocon during opsin biogenesis**. *Biochem J* 2008, **411**:495-506.

92. Wanker EE, Sun Y, Savitz aJ, Meyer DI: **Functional characterization of the 180-kD ribosome receptor in vivo**. *J Cell Biol* 1995, **130**:29-39.

93. Ueno T, Tanaka K, Kaneko K, Taga Y, Sata T, Irie S, Hattori S, Ogawa-Goto K: **Enhancement of procollagen biosynthesis by p180 through augmented ribosome association on the endoplasmic reticulum in response to stimulated secretion**. *J Biol Chem* 2010, **285**:29941-29950.

94. Benedix J, Lajoie P, Jaiswal H, Burgard C, Greiner M, Zimmermann R, Rospert S, Snapp EL, Dudek J: **BiP modulates the affinity of its co-chaperone ERj1 for ribosomes**. *J Biol Chem* 2010, **285**:36427-36433.

95. Blau M, Mullapudi S, Becker T, Dudek J, Zimmermann R, Penczek PA, Beckmann R: **ERj1p uses a universal ribosomal adaptor site to coordinate the 80S ribosome at the membrane**. *Nat Struct Mol Biol* 2005, **12**:1015-1016.

96. • Lakkaraju AKK, Thankappan R, Mary C, Garrison JL, Taunton J, Strub K: **Efficient secretion of small proteins in mammalian cells relies on Sec62-dependent posttranslational translocation**. *Mol Biol Cell* 2012, **23**:2712-2722.

This work explored the range of SRP-independence and Sec62-dependence in mammals, and suggested that the length of the secreted substrate is the main determinant deciding which targeting and translocation pathways will be recruited.

97. Johnson N, Haßdenteufel S, Theis M, Paton AW, Paton JC, Zimmermann R, High S: **The signal sequence influences post-translational ER translocation at distinct stages**. *PLoS ONE* 2013, **8**:e75394.

98. Reithinger JH, Kim JEH, Kim H:: **Sec62 protein mediates membrane insertion and orientation of moderately hydrophobic signal anchor proteins in the endoplasmic reticulum (ER)**. *J Biol Chem* 2013, **288**:18058-18067.

99. Müller L, de Escauriaza MD, Lajoie P, Theis M, Jung M, Müller A, Burgard C, Greiner M, Snapp EL, Dudek J *et al.*: **Evolutionary gain of function for the ER membrane protein Sec62 from yeast to humans**. *Mol Biol Cell* 2010, **21**:691-703.

100. Jermy AJ, Willer M, Davis E, Wilkinson BM, Stirling CJ: **The Brl domain in Sec63p is required for assembly of functional endoplasmic reticulum translocons**. *J Biol Chem* 2006, **281**:7899-7906.

101. Fang H, Green N: **Nonlethal sec71-1 and sec72-1 mutations eliminate proteins associated with the Sec63p-BiP complex from *S. cerevisiae***. *Mol Biol Cell* 1994, **5**:933-942.

102. Feldheim D, Schekman R: **Sec72p contributes to the selective recognition of signal peptides by the secretory polypeptide translocation complex**. *J Cell Biol* 1994, **126**:935-943.

103. Schlegel T, Mirus O, von Haeseler A, Schleiff E: **The tetratricopeptide repeats of receptors involved in protein translocation across membranes**. *Mol Biol Evol* 2007, **24**:2763-2774.

104. Piña FJ, O'Donnell AF, Pagant S, Piao HL, Miller JP, Fields S, Miller EA, Cyert MS: **Hph1 and Hph2 are novel components of the Sec63/Sec62 posttranslational translocation complex that aid in vacuolar proton ATPase biogenesis**. *Eukaryot Cell* 2011, **10**:63-71.

105. Schuldiner M, Collins SR, Thompson NJ, Denic V, Bhamidipati A, Punna T, Ihmels J, Andrews B, Boone C, Greenblatt JF *et al.*: **Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile**. *Cell* 2005, **123**:507-519.

106 • Pfeffer S, Dudek J, Gogala M, Schorr S, Linxweiler J, Lang S, Becker T, Beckmann R, Zimmermann R, Förster F: **Structure of the mammalian oligosaccharyl-transferase complex in the native ER protein translocon**. *Nat Commun* 2014, **5**:3072.

Using a combination of cryoelectron microscopy, and cryoelectron tomography and gene silencing, revealed the *in situ* localization of oligosaccharyl-transferase complex, responsible for cotranslational N-glycosylation. This approach is an appealing tool for further hypothesis-driven functional studies.