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The Back and Forth of Cargo Exit from the Endoplasmic Reticulum

Review

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Endoplasmic reticulum (ER) to Golgi trafficking is an essential step in sorting mature, correctly folded, processed and assembled proteins (cargo) from immature proteins and ER-resident proteins. However, the mechanisms governing trafficking selectivity, specificity and regulation are not yet fully understood. To date, three complementary mechanisms have been described that enable regulation of this trafficking step: ER retention of immature proteins in the ER; selective uptake of fully mature proteins into Golgi-bound vesicles; and retrieval from the Golgi of immature cargo that has erroneously exited the ER. Together, these three mechanisms allow incredible specificity and enable the cell to carry out protein quality control and regulate protein processing, oligomerization and expression. This review will focus on the current knowledge of selectivity mechanisms acting during the ER-to-Golgi sorting step and their significance in health and disease. The review will also highlight several key questions that have remained unanswered and discuss the future frontiers.

Introduction

A cell's ability to sense and respond to its surroundings lies at the heart of almost every aspect of cell function. Central to this ability are the membrane spanning and soluble secreted proteins that serve at the interface between the internal landscape of the cell and its external environment. The folding and maturation of these abundant and diverse proteins is managed by a specialized compartment, the endoplasmic reticulum (ER). Protein folding is not a simple feat: it requires the peptide to achieve its correct tertiary and quaternary structure, as well as undergo numerous post-translational modifications. Thus, the ER is constantly teeming with proteins at all stages of assembly, only a fraction of which are ready to exit the ER for their eventual functions in the later organelles of the endomembrane system.

Once proteins have been correctly assembled, they are packaged into vesicles formed by coat protein complex II (COPII) that transport them to the Golgi apparatus from where they will be sorted to their various destinations in the secretory and endocytic pathway. The ER-to-Golgi trafficking shuttle is the first in the vesicular trafficking network of the endomembrane system. During this step, mature, correctly folded, processed and assembled proteins (cargo) are distinguished from immature proteins and ER residents in order to allow their progress through the secretory pathway. We now have a good understanding of the basal machinery driving creation of the vesicle coat (which involves the formation of an inner shell comprising Sec23 and Sec24 that sorts cargo into ER-derived vesicles and an outer shell comprising Sec13 and Sec31 that promotes coat polymerization) as well as the budding process [1]. The mechanisms governing cargo selectivity and specificity as well as the regulation of ER exit are not yet fully understood, however.

Generally speaking, mature secreted proteins are distinguished from those that should not be released from the ER by three mechanisms (Figure 1). The first is ER retention of immature cargo through their interaction with chaperones and other components of the quality control machinery during the folding and maturation of this cargo. These interactions can prevent entry of immature cargo into COPII vesicles and thus slow down their exit from the ER. The mature cargo is freed from these interactions and can thus enter COPII vesicles in a non-selective manner, often termed 'bulk flow'. The second method is selective uptake, by which, in order to allow efficient export of only mature cargo, some cargos are selectively concentrated by directly or indirectly binding the Sec24 subunit of the COPII complex. ER-resident proteins and immature proteins are not recognized by this system and thus will not exit the ER as efficiently. Finally, retrieval from the Golgi apparatus occurs by capture molecules, such as cargo receptors or the coat protein complex I (COPI) itself, that mediate selective retrieval of immature cargo that has erroneously exited the ER and carry it from the Golgi back to the ER through COPI vesicles.

It should be noted that many ER-resident proteins also use retention and retrieval as a way of reducing the risk of export from the ER. However, we will not discuss these proteins in this review. Depending on their needs during biogenesis (folding, modification and assembly), some secretory proteins rely more on one of these mechanisms to ensure the integrity of their trafficking, whereas others may use various combinations of the three mechanisms to increase fidelity and selectivity. For instance, if both selective uptake and ER retention were not enough to inhibit the escape of immature proteins from the ER, then retrieval can act as a 'fail-safe' mechanism. Hence, it is most likely that the combinatorial nature of the three processes is what safeguards the secretory pathway from forward traffic of proteins that should not leave the ER and enables accurate secretion of only well-folded, mature, secretory proteins. Examples for each of the three mechanisms are abundant, and in this review we will focus on a few prominent cases that demonstrate how each mechanism enables efficient and regulated ER exit.

Mechanisms of ER Retention

Immature cargo proteins have the intrinsic property of being misfolded or misassembled, usually manifested in exposed hydrophobic stretches. This property tags immature cargo for further interactions with chaperones, binding partners or degradation machinery, allowing them additional chances to refold or be sent for degradation. Some components of this machinery together with incompletely folded proteins are thought to form large complexes in the ER, presumably reducing the mobility of these proteins within the ER and the chances for random diffusion into COPII vesicles. This distinction between cargo proteins that are bound or free of ER-processing machineries is one way in which immature proteins can be distinguished from mature ones [2–4].

A very well-studied retention system is the calnexin–calreticulin cycle. In this system, recognition of misfolding is performed by the enzyme UDP-glucose:glycoprotein

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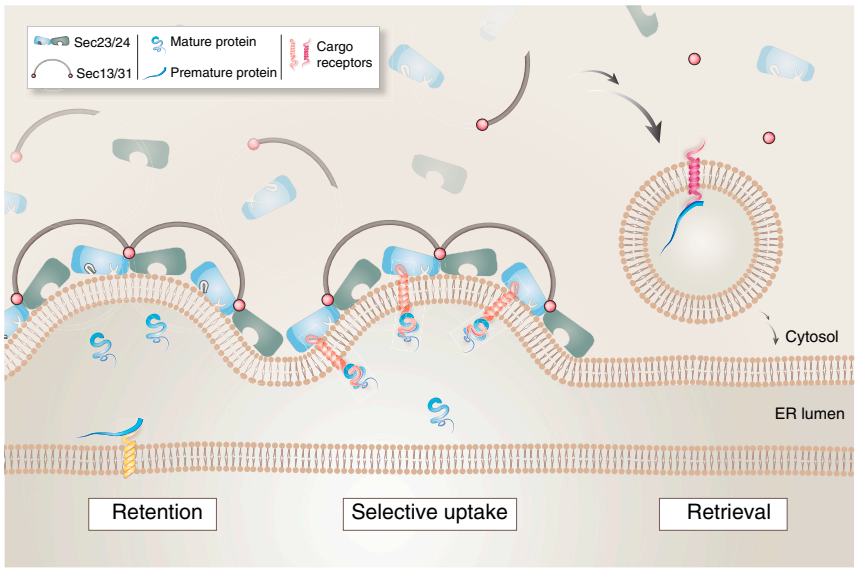

Figure 1. Representation of the three mechanisms used by the secretory pathway to distinguish mature proteins destined for export from misfolded and ER-resident proteins: retention, selective uptake and retrieval.

ER retention: Misfolded proteins are recognized by the ER's rigorous quality control system and retained bound to its components. Mature correctly folded proteins do not interact with chaperones or other ER-resident proteins and can diffuse into COPII vesicles without necessitating interaction with the coat proteins. Selective uptake: COPII coat proteins interact with specific sorting elements held only by proteins destined for secretion. Immature or ER-resident proteins will not be recognized by this machinery. Retrieval: Immature and ER-resident proteins are selectively retrieved and sent back to the ER by COPI vesicles.

glucosyltransferase, which is responsible for detecting the hydrophobic stretches that characterize immature proteins and for glucosylating them [5]. Glucosylated proteins are recognized by either of the two chaperones calnexin or calreticulin. This system negates free diffusion of misfolded proteins by keeping them bound to chaperones until they are correctly folded [6,7].

Another example is specific to membrane proteins, such as LPR6 [8], a metazoan plasma membrane protein involved in Wnt signaling, or the yeast chitin synthase Chs3 [9]. To exit the ER, both proteins must be palmitoylated on a juxtamembranal cysteine. This modification allows for a part of the transmembrane domain that interacts with chaperones to be masked by tilting the entire transmembrane domain relative to the membrane. Loss of contact with chaperones enables the forward trafficking. Lack of palmitoylation leads to retention of the misfolded form and its ubiquitination and degradation, instead of ER exit.

An additional mechanism suggested for retaining misfolded proteins in the ER is compartmentalization of misfolded substrates into ER quality control sites by their selective binding to receptor lectins. These ER quality control sites are enriched for components of the ER-associated degradation (ERAD) machinery and depleted of trafficking regulators, making forward traffic less likely [10].

Mechanisms of Selective Uptake

Exit of vesicles from the ER to the Golgi apparatus occurs at unique spatial subdomains of the ER termed ER exit sites (ERES) [11]. Proteins trafficking forward by the 'bulk flow' mechanism are predicted to simply diffuse into COPII vesicles, where they will be found at a concentration that is in equilibrium with the ER lumen. In contrast, cargos that undergo selective uptake are subject to a concentration step, so that mature cargo in the vesicle is found at concentrations that are higher than in the rest of the ER lumen. This concentration step is mediated by interactions with the Sec24 subunit of the COPII coat itself [12] or by a diverse group of proteins that bridge the interaction between Sec24 and cargo and have been termed cargo receptors. The specificity of selective uptake hinges upon the presence of a sorting signal on the mature cargo, ranging from simple acidic peptides to conformational epitopes and post-translational modifications [13–15].

Even in the simple yeast more than 800 mature proteins must exit the ER. How can Sec24 bind so many clients? Binding diversity is achieved by Sec24 being merely the top of a pyramid, with a base so wide that it would potentially allow the specific binding of hundreds of different proteins. Specifically, cells encode several Sec24 paralogs, with each Sec24 harboring several different binding pockets, and with each binding pocket being able to bind cargo receptors that increase the binding capacity of that pocket, and each cargo receptor having the possibility to engage additional specificity-conferring adaptors. Hence a protein can either bind an adaptor, a cargo receptor or one of the multiple binding proteins created directly by the diversity of Sec24 paralogs and binding pockets. This modularity creates the freedom to generate hundreds of binding opportunities (Figure 2).

Sec24 Variants: The Tip of the Pyramid

Sec24 has a dual role in the COPII complex: in addition to its structural role, it is the cargo selection subunit of the coat and interacts directly with the sorting signals of either cargo or cargo receptors. Most eukaryotes encode several Sec24 paralogs, increasing the diversity of signals that can be recognized by Sec24. For example, in the baker's yeast *Saccharomyces cerevisiae* there are three Sec24 paralogs (Sec24, Sfb2/Iss1 and Sfb3/Lst1) [16], while in mammals there are four (SEC24A,B,C and D) [17]. Indeed, each Sec24 isoform has been shown to mediate ER exit of different proteins. For example, in yeast Sec24 packages the SNARE Sec22, exclusively [12,18], while Sfb3 enables export of the H^+-ATPase Pma1 [16]. In mammals, ER-to-Golgi export of the soluble protein PCSK9 is mediated mainly by SEC24A [19], while the planar cell polarity regulator VANGL2 is sorted exclusively by SEC24B [20] and the two highly related neurotransmitter receptors SERT and GAT1 are differentially dependent on SEC24C and SEC24D, respectively [21,22].

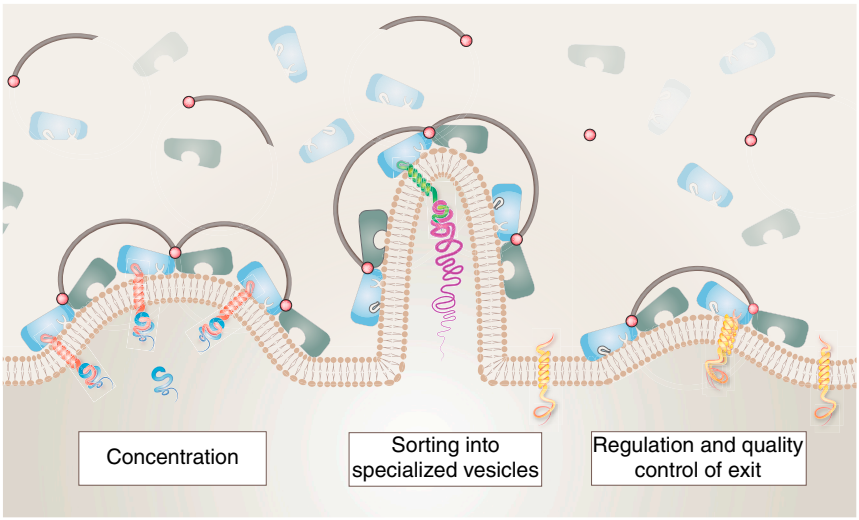

Figure 2. The main advantages of regulated ER exit.

Left: the possibility of concentrating secreted proteins, thus increasing export efficiency. Middle: adaptation of vesicle formation to cargos of special shape or size by interaction with the COPII coat proteins, increasing specificity. Right: regulated traffic enables quality control on maturation, as well as post-translational regulation, thereby increasing accuracy.

Sec24-Binding Sites: One Step Down

The interaction between cargos and the Sec24 coat subunit is mediated through distinct binding sites on the protein surface. Most of the knowledge in this field was gathered from structural studies and experiments performed with the yeast Sec24 protein. In this molecule, three cargo-binding sites have been described to date and are defined as the A, B and C sites. The A site is a hydrophobic pocket found to bind the SNARE Sed5 through an interaction with the YxxxNPF motif [23,24]. The B site is the most diverse site, interacting with acidic motifs such as DxE, LxxLE, and LxxME found on the cargo proteins Sys1, Bet1 and Sed5, respectively [23,25]. The C site interacts with a conformational epitope of the SNARE Sec22 [14,23,25]. It is assumed that the Sec24 homologs in mammals also possess at least some of these binding sites and that additional binding sites may still exist on Sec24 itself or on its paralogs. Thus, binding sites can triple or more the options for binding a specific Sec24 isoform.

Cargo Receptors: From the Bottom Up

Despite the wide diversity of binding sites offered by the Sec24 family members, not all cargo proteins can interact directly with Sec24. This is because some proteins are confined to the lumen of the ER and cannot access the cytosolic leaflet of the ER on which Sec24 assembles. In addition, there are proteins with modifications on their cytosolic segments that preclude Sec24 binding or that were constrained in evolving a direct Sec24-binding site. These proteins can interact with Sec24 through a diverse group of proteins that mediate the interaction between Sec24 and cargo, termed cargo receptors [26]. Extensive characterization of cargo receptors has been provided from studies in yeast and cargo specificity has been identified for many of these cargo receptors. For example, Erv29 sorts soluble proteins like pro-α-factor and carboxypeptidase Y [27], the p24 proteins promote efficient export of glycosylphosphatidylinositol (GPI)-anchored proteins [28,29], Erv14 mediates export of proteins with a long transmembrane domain [30,31], Erv26 promotes the export of Golgi mannosyltransferases as well as pro-alkaline phosphatase [32,33], and Emp46/Emp47 are required for the export of some glycoproteins [34].

One example for how binding to a cargo receptor can help differentiate between mature and immature cargo proteins comes from studying the mammalian cargo receptor ERGIC-53, which binds a wide variety of glycoproteins and concentrates them into COPII vesicles. To ensure that ERGIC-53 interacts with only properly folded and glycosylated proteins, the recognition of cargo by ERGIC-53 relies on interactions with both N-linked carbohydrates as well as structural motifs. For example, its interaction with procathepsin Z is mediated by a β-hairpin loop next to the N-linked carbohydrate [15].

The number of cargo receptors and their substrate range known to date cannot account for even a small fraction of all exported proteins from the ER. This could imply that an enormous number of cargo receptors await discovery, that Sec24 is capable of directly binding hundreds of proteins, or that active uptake is the mechanism of choice for only a very specific group of proteins.

Adaptors: The Base of the Pyramid

An additional layer of diversity is enabled by ‘adaptors’, a group of auxiliary proteins that bind cargo receptors and increase their recognition repertoire to a wider set of cargos. A prominent example is the differential interaction of ERGIC-53 with two auxiliary proteins, ERp44 and MCFD2, in order to promote ER export of the immunoglobulin IgM or coagulation factors V and VIII, respectively [35,36].

Mechanisms of Retrieval

Retrieval is an essential step in controlling specific export of mature proteins and provides a safety net to retrieve substrates that have left the ER, despite being ER residents or immature proteins. Retrieval, like selective uptake, can occur if substrates bind the coat subunit of the COPI coat directly or through dedicated cargo receptors [37]. For example, during the proper hetero-octameric assembly of the Kir6 inward rectifier potassium channel, the arginine-based retrieval signal of each subunit is masked and allows the assembled channel to traffic to the cell surface. Monomeric subunits, whose retrieval signal is not masked, will be efficiently recognized at the Golgi apparatus and retrieved via COPI vesicles [38,39]. GABA receptors [40] and kainite receptor [41] use the same retrieval mechanism.

Retrieval signals can also occur in transmembrane regions. For example, the two subunits of the yeast iron transporter, Ftr1 and Fet3, must be assembled in order to traffic through the secretory pathway. Monomers of Fet3 will exit the ER but be retrieved from the Golgi by the retrieval

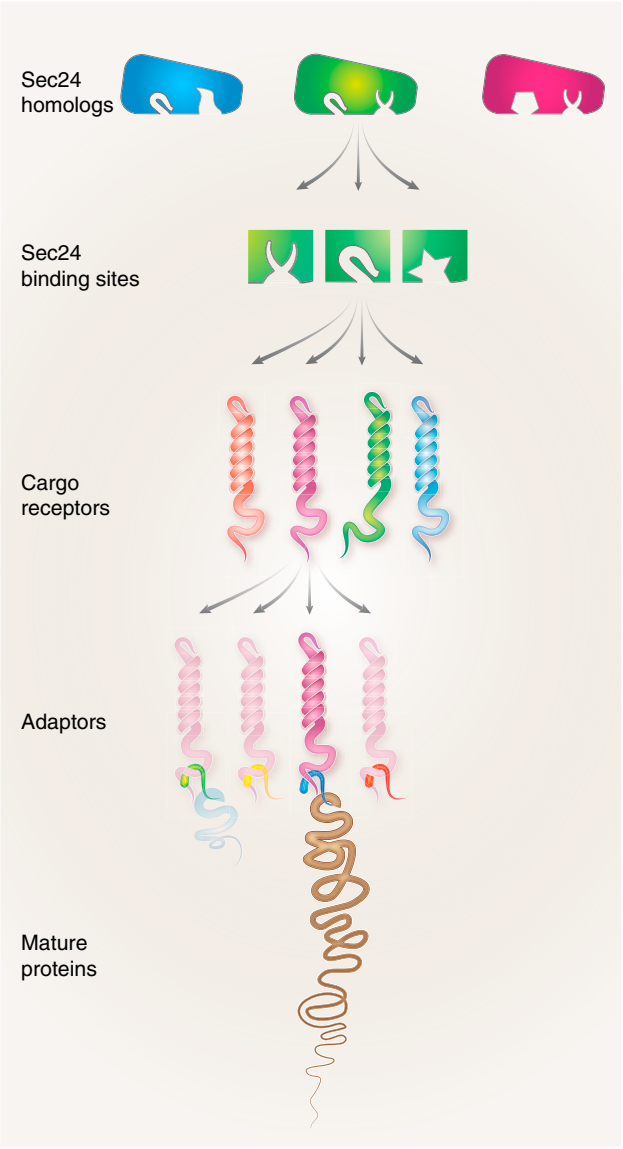

Figure 3. The hierarchical pyramid of selective uptake.

Specific binding of the COPII machinery to a wide spectrum of secreted proteins is enabled through a hierarchy of interactions. The upper layer represents the Sec24 homologs, each of which harbors several binding pockets as a second layer of binding possibilities, with a third layer being provided by the cargo receptors which can interact with different adaptors to enable a broad range of specific interactions with different cargo proteins. Each layer in this pyramid can directly bind a cargo protein, creating specificity and diversity.

receptor Rer1. Correct assembly with Ftr1 masks the Rer1 interaction region in the Fet3 transmembrane region and releases it from recycling between the ER and the Golgi [42]. Similarly, a transmembrane retrieval signal is used in the NMDA receptor [43,44].

Two Steps Forward, One Step Back — Working Together to Ensure Accurate Forward Traffic

Often selective uptake and retrieval work together to prevent the export of unassembled subunits and promote the export of fully assembled complexes. For example, only the properly oligomerized IgM antibody is selectively recruited into COPII vesicles by ERGIC-53. However, in cases where monomeric subunits of IgM make it out of the ER, ERGIC-53 and the auxiliary factor ERp44 bind to these monomers in the Golgi and take them back to the ER [36].

Another example pertains to GPI-anchored proteins that have a glycolipid anchor attached to their carboxyl terminus. The remodeling steps of this lipid anchor that occur in the ER are a prerequisite for concentration and forward traffic via attachment to the p24 cargo receptor complex [45] (composed of Emp24, Erv25, Erp1, and Erp2 in yeast [46,47]). Interestingly, in yeast the p24 complex has a higher affinity for remodeled GPI anchors and so in the ER attaches actively to the fully remodeled GPI-anchored proteins. However, in the Golgi, where remodeled GPI-anchored proteins continue to be rapidly forward trafficked, p24 attaches to the non-remodeled chains and actively promotes retrieval through interactions with the COPI coat [47].

Advantages of Regulated ER Exit

Regardless of how selectivity is achieved, it is important not only for distinguishing between mature and immature proteins for secretion, but also for sorting cargo into unique exit sites and for several aspects of post-translational regulation (Figure 3).

Sorting into Specialized Vesicles

Due to the diverse nature of cargo, different physicochemical parameters may be relevant during their maturation and ER export. In yeast three distinct populations of ER exit sites (ERESs) were identified, each containing different cargo types: soluble cargos like pro-α-factor, transmembrane proteins like the general amino acid transporter Gap1, or GPI-anchored proteins. Each ERES probably contains conditions that are optimal for its own cargo's maturation process. For example, in GPI-anchored proteins the fully remodeled anchor confers specific association with membrane microdomains that are enriched in sterols and sphingolipids [48,49]. Interestingly 20–30% of GPI-anchored proteins and transmembrane proteins were found in the same ERES and the same COPII vesicles. This implies that interactions with specific COPII coats and adaptors serve to concentrate cargo in vesicles of choice but do not unequivocally exclude cargo from imperfect vesicles [46].

Another group of clients requiring specialized vesicles are cargos of unique dimensions. Most COPII vesicles are approximately 60–80 nm in diameter, yet there are some much larger known cargos like the 300–400 nm procollagens. Thus, mechanisms exist to enlarge COPII vesicles when required. Specifically, monoubiquitylation of Sec31 by the ubiquitin ligase CUL–KLHL12 promotes the formation of large COPII coats that can accommodate unusually shaped cargo [50]. Such cargo has also an unusual selective loading mechanism into COPII vesicles: TANGO1, an integral membrane protein localized to ERESs, loads collagen VII into transport carriers without following the cargo into the vesicle itself. It has been postulated that an interaction between a cytoplasmic region of TANGO1 and Sec23/Sec24 delays recruitment of the outer layer Sec13/Sec31 subunits of the COPII coat and enables essential architectural modifications to be made [51].

Post-Translational Regulation

Cells must deal with rapidly changing environmental, developmental and internal cues. As such, having mechanisms to regulate cell-surface expression of proteins without the need for the lengthy cycle of transcription and translation gives flexibility and an advantage in reaction times. Hence, trafficking is an important component of post-transcriptional regulation. Use of trafficking enables large amounts of proteins to be synthesized yet retained in an inactive state by ER retention, degradation or retrieval. When cells require the function of the protein, rapid cell-surface expression can be achieved.

One of the examples that best demonstrates the importance of trafficking in post-translational regulation is the selective retention of the ER-membrane-bound transcription factor ATF6. ATF6 is the activator of one of the arms of the unfolded protein response (UPR) that reacts to ER stress and hence must be kept in an inactive state during normal cell growth. Retention of ATF6 in the ER is achieved by association with the most prevalent lumenal chaperone, BiP. When the ER is stressed, a dissociation trigger that has not yet been characterized allows ATF6 to be trafficked to the Golgi [52]. In the Golgi, non-selective cleavage releases the amino-terminal cytoplasmic domain of ATF6, which is now free to diffuse into the nucleus and act as a transcription factor to induce expression of ER chaperones, such as BiP and GRP94. Hence, ER retention in this example is a powerful mechanism for regulating cellular physiology [53].

Well-studied examples of regulated secretion come from proteins carrying a carboxy-terminal arginine-based retrieval signal. When this signal is masked by interactions with 14-3-3 proteins, the proteins carrying this retrieval signal cannot interact with the COPI vesicle proteins, leading to efficient cell-surface expression. Downregulation of the 14-3-3 proteins leads to ER retrieval and rapid downregulation of cell-surface expression. The affinity of 14-3-3 proteins for their interaction partners is significantly increased by phosphorylation. Thus, rapid phosphorylation of 14-3-3 client proteins can cause an immediate and dramatic increase in forward traffic [54].

Another group of proteins that post-translationally regulate trafficking of cargo are the PDZ domain proteins. Specifically, interaction of the PDZ domain with cargos bearing a PDZ-binding domain often masks a carboxy-terminal retrieval signal and enables forward traffic. One well-studied substrate is the NR1 subunit of the NMDA receptor. NR1 exists in eight alternatively spliced isoforms, only some of which bear a retrieval sequence and a PDZ binding domain. By this mechanism the activity and availability of PDZ proteins, alongside alternative splicing, affect the types and amounts of synaptic receptors on the plasma membrane [55]. In a similar mechanism, the di-leucine signal in synaptic adhesion-like molecule 1 (SALM1) is thought to be masked by interactions with PDZ domain proteins [56], and proTGF-α trafficking is modulated by binding to the ER-resident PDZ domain protein, TACIP18 [57].

Another example is the opposing forces imposed by binding of glutamate transporter-associated protein 3-18 (GTRAP3-18) and the reticulon RTN2B to the glutamate transporter EAAC1. Both proteins can separately and independently form complexes with EAAC1, but interaction with GTRAP3-18 prevents ER-to-Golgi traffic, whereas interaction with RTN2B facilitates this traffic [58,59]. Thus, differential expression of the two proteins can dramatically alter cell-surface expression of the glutamate transporter in a rapid and efficient manner.

Post-translational regulation can also occur by modifications of the cargo proteins. For example, Chs2 is a yeast chitin synthase that is active at the bud neck during late mitosis. During metaphase Chs2 is phosphorylated by cyclin-dependent kinase 1, which reduces its interaction with Sec24 and causes it to accumulate in the ER. When its activity is required, during mitosis, it is dephosphorylated by the Cdc14 phosphatase, stimulating its interaction with Sec24 and its rapid export to the bud neck [60].

Lessons on Specificity of Protein Secretion from Mammalian Models and Human Diseases

As discussed above, many of the examples of specificity in trafficking come from studies of cells where post-translational regulation is a central part of their function — neurons. Hence, many of the studies focus on cell-surface expression of various channels and transporters that modulate neuronal function [61,62]. An additional source of knowledge on how specificity is created in protein secretion arises from studies of human diseases and animal models. For example, the link between ERGIC-53, one of the most well-studied cargo receptors, and its cargo, coagulation factors V and VIII, arose from linkage analysis of individuals with combined deficiency of coagulation factors [63]. Similarly, one of the adaptors of ERGIC-53, MCFD2, was discovered by linkage analysis of similar patients that had normal levels of ERGIC-53 itself [35]. The discovery of MCFD2 demonstrated that auxiliary proteins can interact with cargo receptors to promote specific protein export.

Studies on deletion mutants of *SEC24* isoforms in mice helped uncover the specific dependencies of proteins on SEC24 paralogs. While deletion of SEC24C or SEC24D caused an early embryonic lethal phenotype [64], SEC24B mutations enabled embryos to develop to term; however, these mice died immediately following birth. Interestingly, death was found to be a result of craniorachischisis, a severe birth defect of an open neural tube. Similar defects have previously been shown to arise from mutants in the planar cell polarity pathway. Indeed it could be shown that one of the key components of this pathway, VANGL2, is dependent specifically on SEC24B for its proper ER-to-Golgi traffic [20,65]. Most probably many more proteins require SEC24B specifically for ER exit and future work to uncover them may lead to deeper understanding of the mechanisms guiding specificity and promiscuity in SEC24 interactions.

Unlike deletions of the other three isoforms, mice carrying the *SEC24A* null mutation displayed a much milder effect. The mutant mice exhibited normal development and survival, but showed about 40% reduction in plasma cholesterol. Indeed, the cargo responsible for this phenotype is PCSK9, a secreted protein that binds low density lipoprotein receptor and promotes its endocytosis and degradation [19].

Although many of these studies focus on a single substrate, the hope is that, in the future, identification of the various substrates will enable a more complete understanding of the underlying determinants that enable specificity.

The Next Frontiers

Although specific examples of retention, selective uptake, and retrieval are abundant, each report emphasizes different key elements in the process and very few deal with the complexity of all three mechanisms functioning together.

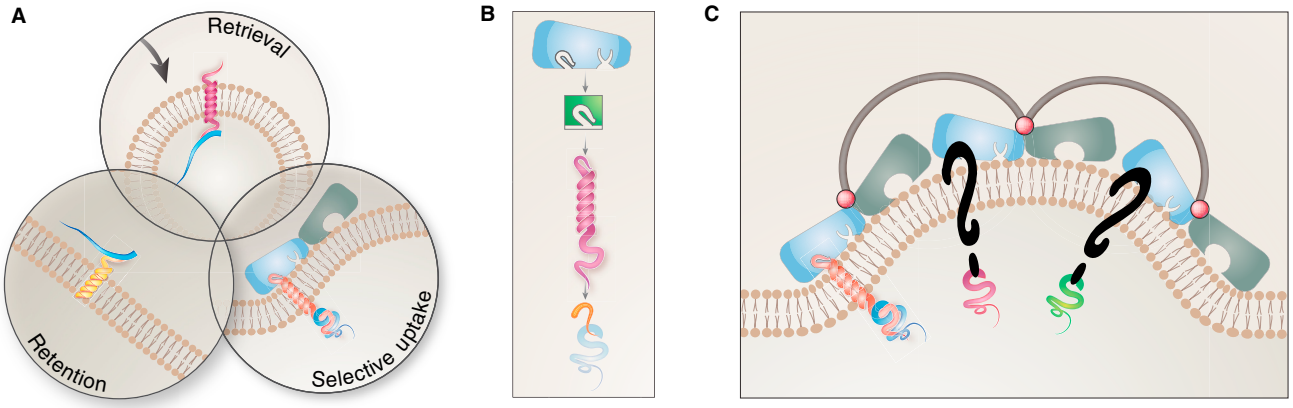

Figure 4. Major open questions in the field.

(A) We still lack a systematic and thorough understanding of which proteins use each mechanism to enable regulated ER export and the extent of the overlap between the various mechanisms. (B) For proteins using regulated uptake we still need to uncover which COPII coat protein, cargo receptor and adaptor are required for these proteins to exit the ER. (C) Discovery of additional proteins that regulate ER export will provide a full picture of how this step occurs. There are probably additional cargo receptors, adaptors and Sec24-binding sites to be found.

Hence, a systematic understanding of when, where and how these three prominent mechanisms function to regulate ER traffic is still lacking. In our eyes, there are very exciting challenges for the field (Figure 4). On the one hand, there are still fundamental unresolved mechanistic questions that must be addressed, such as understanding the molecular bases of the cargo receptor operation mode, defining the contribution of oligomeric cargo receptors to ER export and understanding whether receptors cycle constitutively or cargo binding stimulates packaging of receptors into COPII vesicles. On the more general scale, systematic studies uncovering the client base for each of the three regulation mechanisms — retention, selective uptake and retrieval — and how they overlap to ensure accurate secretion will be an essential step in driving this field forward. In addition, it is essential now to start understanding the rules underlying recognition of a specific substrate by a given pathway and characterizing the exact proteins in that pathway. For example, for a protein that exits the ER through selective uptake, does it use an adaptor? Does it use a cargo receptor and which one? Which Sec24 isoform does it require and which binding sites can it use? Finally, there is still a need to discover new Sec24-binding sites as well as proteins performing and regulating each step (specific retention proteins, kinases/phosphatases that modulate binding of cargo to trafficking machinery, and additional cargo receptors and adaptors). Future work should therefore aim to put each trafficking specificity step in the context of all other processes occurring around it and use each finding to generalize the rules governing pathway and substrate selection [31,66]. As more work is performed and with each new insight into the system, our ability to find common elements guiding recognition and selection should increase. In the future, such efforts should allow us to map the molecular mechanisms that govern specificity and selectivity in regulated ER-to-Golgi traffic.

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