Signals for COPII-dependent export from the ER: what's the ticket out?

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Export of many secretory proteins from the endoplasmic reticulum (ER) relies on signal-mediated sorting into ER-derived transport vesicles. Recent work on the coat protein complex II (COPII) provides new insight into the mechanisms and signals that govern this selective export process. Conserved di-acidic and di-hydrophobic motifs found in specific transmembrane cargo proteins are required for their selection into COPII-coated vesicles. These signaling elements are cytoplasmically exposed and recognized by subunits of the COPII coat. Certain soluble cargo molecules depend on receptor-like proteins for efficient ER export, although signals that direct soluble cargo into ER-derived vesicles are less defined.

The eukaryotic secretory pathway sorts and delivers a tremendous variety of proteins to their proper intracellular locations. Delivery proceeds through a series of directed membrane translocation, membrane budding and membrane fusion events that guide secretory proteins to their ultimate destination. Current evidence indicates that signaling elements within secretory proteins are deciphered by the intracellular transport machinery for proper routing. The identification of these specific sorting signals and mechanisms of their recognition are of fundamental importance to cell biology.

In the case of protein sorting during vesicle formation, elegant studies on clathrin-dependent endocytosis of the LDL-receptor uncovered one of the first such sorting signals [1]. A short cytoplasmic sequence on the LDL-receptor (DPNY) was found to target proteins into clathrin-coated pits. Further studies demonstrated that this signal binds directly to a clathrin adaptor protein during endocytosis and provided a cogent model for how specific cargo can be selected into coated vesicles. With the subsequent discovery of other cytosolic coat protein complexes, searches for signals that direct cargo proteins into other types of transport vesicles were initiated. Some such signals have been identified, and an emerging principle suggests that coat protein complexes bind to specific signals to transport proteins out of a given membrane compartment [2]. However, the complete set of sorting signals and the manner by which they act are not understood. This review will examine signals that mediate ER export and the molecular mechanisms by which an ER-specific coat complex uses these signals in selective export. Although a comparison with clathrin studies might be a good starting point, there are unique mechanisms underlying protein export from the ER.

Overview of the early secretory pathway

The coat protein complex II (COPII) forms transport vesicles from the ER and collects the appropriate cargo proteins into these vesicles. Certain signals have been implicated in COPII-dependent export; however, there is an extraordinary diversity in the cargo selected by the COPII coat and many exported proteins do not contain known sorting signals. Moreover, the ER export machinery has to deal with an additional complexity, introduced by the process of quality control, whereby export cargo is apparently retained and/or not recognized by the COPII coat until fully folded and assembled [3]. Forward transport of folded secretory proteins from the ER is also balanced by a retrograde transport pathway that depends on an additional coat complex termed COPI. The COPI coat is molecularly distinct from COPII and is thought to form retrograde-directed vesicles from Golgi membranes and pre-Golgi intermediates [2]. Retrograde transport serves to recycle components needed for ER-vesicle formation and to retrieve escaped ER-resident proteins. Studies show that bidirectional transport between ER and Golgi compartments is very dynamic [4], but ultimately biosynthetic secretory cargo advances, whereas resident proteins of the early secretory pathway make no net headway (Fig. 1). The COPII coat acts in a crucial sorting event during export from the ER; therefore, to understand this process, we first need to appreciate how the COPII coat assembles on the surface of ER membranes.

Assembly of the COPII coat

The COPII coat consists of three proteins (Sar1, Sec23–Sec24 complex and Sec13–Sec31 complex) that are sequentially recruited to the ER membrane surface (Fig. 2). Sar1 is a small 21-kDa GTPase, whereas Sec23–Sec24 and Sec13–Sec31 are large heteromeric protein complexes. The Sar1 GTPase cycle is thought to regulate coat assembly and disassembly. To assemble the COPII coat, membrane-bound Sar1–GTP binds to Sec23–Sec24 that in turn attracts Sec13–Sec31 [5]. Activation of Sar1 to Sar1–GTP is catalyzed by a membrane-bound GDP–GTP exchange factor, Sec12, which is localized to the ER [6]. Thus, COPII assembly is restricted to the ER through localized production of Sar1–GTP. Conversely, hydrolysis

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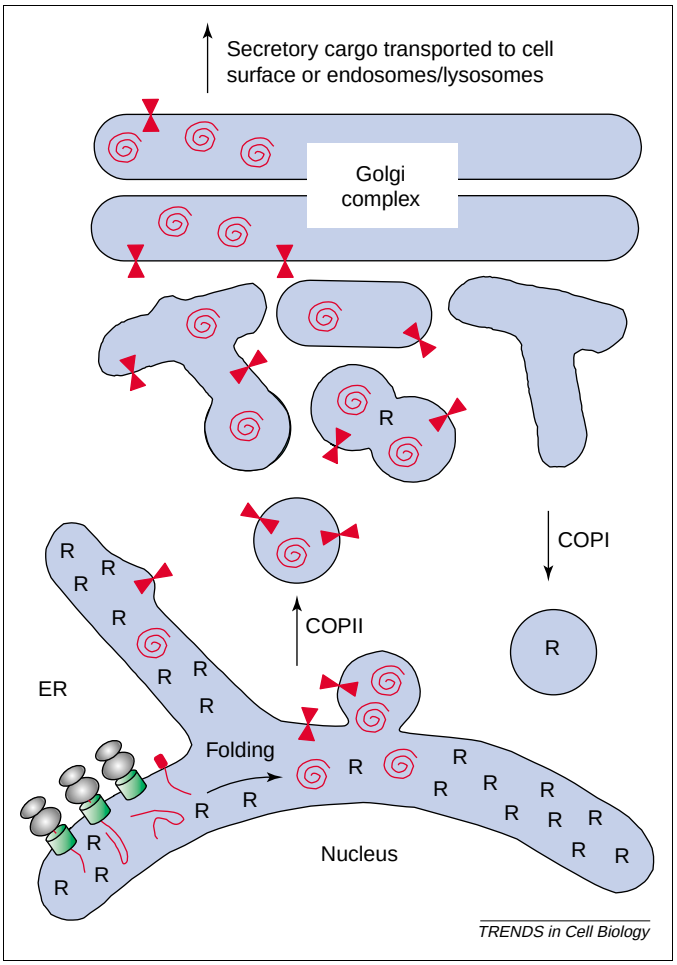

Fig. 1. Coat proteins in organization of the early secretory pathway. Nascent secretory proteins (shown in red) are synthesized and folded by ER-resident proteins (designated 'R'). Fully folded integral membrane and soluble secretory cargo molecules depart the ER in anterograde-directed vesicles produced by the COPII coat. Retrograde-directed vesicles formed by the COPI coat recycle vesicle proteins needed for anterograde transport and retrieve resident proteins that have escaped the endoplasmic reticulum (ER). This dynamic cycling process allows secretory cargo to advance in an anterograde direction, whereas residents remain localized to the early secretory compartments.

to produce Sar1–GDP releases coat subunits from budded vesicles and reverses the assembly process. In the disassembly pathway, the Sec23 subunit stimulates the rate of GTP hydrolysis by Sar1 [7], and, more recently, it has been shown that Sec23 activity towards Sar1 is further stimulated by recruitment of Sec13–Sec31 [8]. Therefore, assembly of the entire COPII coat produces an intrinsically unstable structure, although the coat is thought to somehow persist long enough for vesicle budding to occur [9].

Reconstitution experiments demonstrate that this minimal set of COPII proteins (Sar1, Sec23–Sec24 and Sec13–Sec31) is sufficient to form coated vesicles from synthetic liposomes when nonhydrolyzable GTP analogs are supplied to the reaction. Therefore, it is the polymerization of these COPII subunits on the surface of the ER that is thought to drive membrane deformation [10]. A dependence on nonhydrolyzable forms of GTP in reconstituted budding reactions suggests that additional factors might regulate the COPII assembly–disassembly cycle during export from ER membranes.

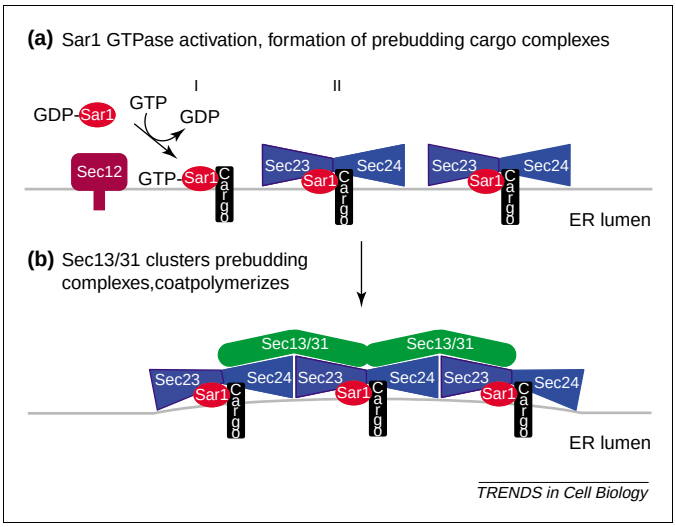

Fig. 2. Model for coordinated assembly of the COPII coat and cargo selection. (a) Sar1 is activated to Sar1–GTP by an ER-localized exchange protein, Sec12 (I). Membrane-bound Sar1–GTP transiently associates with export cargo before assembly into prebudding Sar1–Sec23–Sec24–cargo complexes (II). (b) Prebudding cargo complexes are clustered by the outer layer Sec13–Sec31 protein (green). Polymerization of the COPII coat causes deformation of the endoplasmic reticulum (ER) membrane.

The COPII coat selects export cargo into ER-derived transport vesicles

Experimental evidence demonstrates that certain cargo, in order to be included into COPII vesicles, possess a binding affinity for subunits of the coat. More specifically, pre-budding complexes consisting of Sar1–GTP and Sec23–Sec24 bound to cargo can be isolated under conditions that preserve the Sar–GTP-bound configuration [11,12]. Based on these findings, a model integrating cargo selection and COPII vesicle formation can be constructed as shown in Fig. 2. In this model, pre-budding complexes of Sar–Sec23–Sec24–cargo form on the surface of ER membranes. These pre-budding cargo complexes are then gathered by the Sec13–Sec31 complex into nascent vesicles to extract specific cargo from the ER. Presumably, the Sar1 GTPase is regulated in a manner to allow for productive incorporation of pre-budding cargo complexes into the polymerized coat before hydrolysis of bound GTP. Structural studies on a Sar1–Sec23–Sec24 prebudding complex reveal that the Sec23 subunit shares an extensive binding surface with Sar1 and also supplies an arginine residue to the active site of Sar1 in stimulating GTP hydrolysis. Moreover, the structure of the prebudding complex is 'bowtie-shaped' and forms a concave surface that apparently faces the ER membrane. Therefore, domains of Sar1 and Sec23–Sec24 could well be available for binding to integral membrane cargo proteins [13].

ER export signals

Based on these findings, it has been hypothesized that ER-export cargo possesses surface residues or sequence motifs that are bound to Sar1–Sec23–Sec24 pre-budding complexes directly or indirectly [11,12]. What are these signals and how are they recognized? Recent studies have identified specific signals on transmembrane cargo to support this hypothesis, and structural studies are closing in on precise contacts between cargo and coat. For soluble

secretory cargo proteins that cannot be bound directly by coat subunits, there is no consensus, but evidence suggests that transmembrane receptors might link certain lumenal cargo to COPII.

Signals in transmembrane cargo

Transport of the vesicular stomatitis virus glycoprotein (VSV-G) has served as a model for study of secretory protein folding and export from the ER. VSV-G, a type I transmembrane protein that traffics to the cell surface, is abundantly expressed in VSV-infected cells and concentrated into ER-derived transport vesicles [14,15]. VSV-G possesses a cytoplasmically exposed C-terminal tail sequence of 29 residues that is required for transport from the ER. Within this tail sequence, a conserved *YTD IEM* motif, found in other secreted type I transmembrane proteins, is necessary for efficient export of VSV-G from the ER [15,16]. Mutation of any one of the underlined consensus residues results in a VSV-G protein that assembles into a normal homotrimer but is only very slowly exported from the ER. Furthermore, transfer of the YXDXE sequence to a transport-neutral membrane protein accelerates its export rate, although it should be mentioned that this rate is slower than the rate of wild-type VSV-G transport. The so called di-acidic sequence (DXE) motif contained within the VSV-G tail sequence is found in many other secretory proteins (see Table 1) that are efficiently exported from the ER, including the Kir2.1 potassium channel protein [17] and the yeast membrane proteins Sys1p and Gap1p [19,20]. Moreover, Sys1p depends on its di-acidic residues for direct binding to Sec23–Sec24 [18] and Gap1p requires its di-acidic motif to form pre-budding complexes with Sar1 and Sec23–Sec24 [19].

However, there are many other membrane proteins that are efficiently exported from the ER but do not contain apparent di-acidic motifs. Therefore, it seems this is not the only ticket for export from the ER, and other types of transport signals have been identified in membrane cargo that exit the ER. These generally consist of a pair of bulky hydrophobic residues and have been described as di-aromatic or di-hydrophobic motifs. For example, the membrane protein ERGIC53, which cycles between the ER and Golgi compartments, has been well studied. This type I transmembrane protein possesses a cytoplasmic tail sequence of 16 residues that is required for proper localization. More specifically, a conserved pair of aromatic residues at the extreme C-terminus of ERGIC53 is necessary for transport from the ER [20]. There is some flexibility in this signal as other bulky hydrophobic amino acids can substitute for this C-terminal signal [21]. There is also evidence for a role for these terminal residues in binding to the COPII subunits [20,21]. ERGIC53 homologs in yeast also possess bulky hydrophobic residues at their C-termini (LL) that are required for export from the ER and proper localization. Furthermore, when bulky hydrophobic residues are placed at the C-terminus of a transmembrane reporter protein, transport to the Golgi is accelerated [21,22], although not to rates observed for endogenous ERGIC53.

An additional conserved ER export signal has been identified more recently in the tail sequence of the ERGIC53 family of proteins [23]. This tyrosine-containing motif is ~12 amino acids from the C-terminal signal (see Emp46p in Table 1). Both motifs are required for assembly into COPII pre-budding complexes and for ER export. Other di-aromatic motifs (FF, YY or FY) are found in a similar position in membrane proteins that exit the ER such as the p24 family of proteins [24,25] and the Erv41–Erv46 complex [26]. Interestingly, many of these proteins, including ERGIC53 and VSV-G, form oligomeric complexes, such that a given exported protein would presumably display multiple signals to the COPII budding machinery. Indeed, other reports suggest that multiple signals are needed for efficient export of the Can1p arginine permease [19], the Erv41–Erv46 complex [26] and an ATP-binding cassette transporter protein, Yor1p [27]. A requirement for multiple signals in secretory proteins might be an important element in ER quality control. One might envisage that a single export motif contained on unfolded or unassembled subunits of protein oligomers would possess weak binding affinities for coat subunits, whereas folded and assembled protein complexes would present a combinatorial signal with a higher binding affinity for coat subunits. A requirement for combinatorial signals could operate concomitantly with the ER-retention machinery that retains unfolded proteins [3] to efficiently exclude unassembled oligomers from ER-derived vesicles. Such a mechanism might also explain why transfer of short linear export motifs to transport-neutral species accelerates their exit from the ER but does not appear to confer the 'original' transport rates [15,21,22]. Perhaps appending ER exit signals to proteins whose oligomerization status can be controlled could test this model.

Table 1. Characterized ER export signals

Export signal	Protein	Refs
Di-acidic motifs		
IYTDIEMNRLGK (−1)	VSV-G	[15,16]
ANSFCYENEVAL (−45)	Kir2.1	[17]
QSPIQLKDLESQI (−1)	Sys1p	[18]
AEKMDIDTGR (−34)	Gap1p	[19]
Di-hydrophobic motifs		
YIMYRSQQEAAAKKFF (−1)	ERGIC53	[20]
YYMFRINQDIKKVKLL (−1)	Emp46p	[23]
YLRRFFKAKKLIE (−1)	p24δ1	[25]
YQPDDKTKGILDR (−1)	Erv41p	[26]
KLFYKAQRSIWGKKSQ (−1)	Erv46p	[26]

Underlined residues are required for export from the endoplasmic reticulum (ER). Numbering is from the C-terminal end, where the terminus corresponds to −1.

Sec24 proteins 'collect the tickets'

Several lines of evidence indicate that a family of Sec24 proteins functions in cargo recognition. Furthermore, the presence of multiple Sec24 homologs appears to expand the variety of cargo that must be efficiently exported from the ER. Yeast cells express two additional Sec24-like proteins, termed Lst1 and Iss1. Higher eukaryotes are endowed with at least four Sec24 isoforms [28]. In yeast, the Lst1 subunit is not essential for COPII-dependent export but is required for efficient export of specific transmembrane cargoes from the ER [29,30]. Both

Sec23–Sec24 and Sec23–Lst1 proteins can be incorporated into a continuous COPII structure, suggesting that heterogeneity in the coat could increase the variety of cargo accommodated by a COPII coated vesicle [30]. More recently, a functional sorting assay demonstrated that both Sec23–Sec24 and Sec23–Lst1 can function independently in assembly of COPII coats; however, the spectrum of cargo packaged into vesicles synthesized with Sec23–Sec24 was quite distinct from those generated with Sec23–Lst1 [31]. These observations, coupled with the fact that Sec23–Sec24 displays binding affinities for both di-acidic [18] and di-hydrophobic motifs [20,25,32], support a direct role for Sec24 in cargo recognition. Precisely how Sec24 proteins recognize export signals has not been determined. If one considers that multiple export signals have been identified and that oligomeric cargo might present a combinatorial signal, Sec24 proteins might possess distinct recognition sites for each signal. Alternatively, Sar1 might contribute to cargo recognition through direct association with export signals to form stable prebudding complexes. Interactions between Sar1 and export cargo have been documented [12,26,32,33], although the affinity and specificity of these associations are not well characterized. Additional binding studies with isolated coat subunits and structural analyses of cargo bound to prebudding Sar1–Sec23–Sec24 complexes should further define these molecular arrangements.

Export signals in soluble secretory cargo

Up to this point, the export signals considered have focused on transmembrane cargo proteins, which are directly accessible to COPII subunits. However, there are a variety of soluble secretory proteins that are efficiently exported from the ER and cannot directly contact the COPII coat. Two non-exclusive models, known as the 'bulk flow' and 'receptor-mediated' export models, have been described in studies addressing export of soluble cargo from the ER (Fig. 3).

First, a passive or 'bulk flow' process [34] appears to operate in the export of amylase and chymotrypsinogen from the ER of pancreatic exocrine cells [35]. Concentration of these soluble secretory proteins was not detected in COPII-coated buds but was observed in tubular structures apparently en route to the Golgi complex. A concentration by exclusion model has been proposed to explain this result such that, after soluble proteins exit the ER, cargo is excluded from retrograde-directed COPI vesicles that bud from the observed tubular transport structures [35]. For such a mechanism to operate, some form of cargo aggregation in the ER might serve a crucial role in release of soluble cargo from ER quality-control machinery and possibly in exclusion from COPI vesicles that bud from post-ER structures. If true, sequences that promote cargo aggregation or oligomerization might serve as ER-exit signals in the export of soluble cargo from the ER. Such exit signals could be molecularly defined and potentially transferred to a transport-neutral protein in a test of this model.

Second, the receptor-mediated model hypothesizes that export of soluble cargo from the ER is an active process that concentrates cargo into ER-derived vesicles

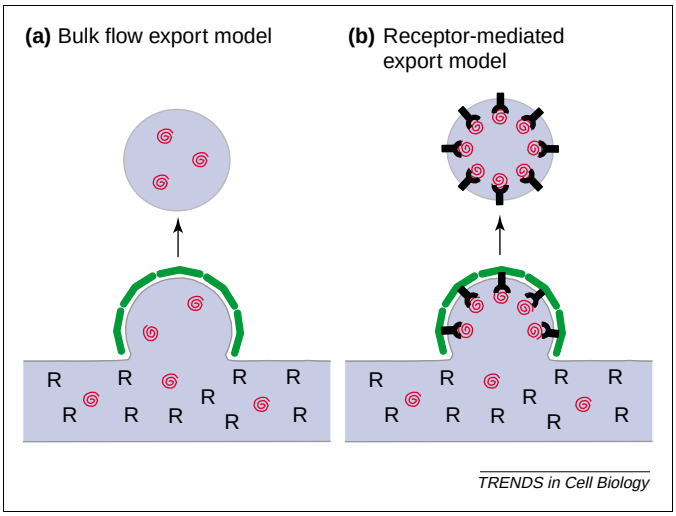

Fig. 3. Bulk-flow and receptor-mediated models for export of soluble secretory cargo from the endoplasmic reticulum (ER). (a) In the bulk-flow model, soluble cargo molecules depart in vesicles at a concentration equal to that found in the ER lumen. (b) The receptor-mediated export model results in concentrative sorting of soluble cargo during vesicle formation and relies on receptor-like proteins to link cargo to the membrane coat complex. In both models, ER-resident proteins (designated 'R') might not be efficiently packaged into ER-derived vesicles owing to the effect of retention and/or exclusion mechanisms.

[11,19,36]. In this model, transmembrane cargo receptors would be needed to link lumenal cargo to the COPII coat. Genetic and biochemical studies suggest that the membrane proteins ERGIC53, the p24 proteins and Erv29p cycle between the ER and Golgi compartments and act as such receptors [37–40]. In the case of ERGIC53, reports indicate that it functions as a transport receptor for a subset of soluble glycoproteins, including blood coagulation factors and cathepsin-Z [37,38]. The lumenal domain of ERGIC53 displays lectin activity [41], and binding between ERGIC53 and the cargo protein cathepsin-Z is carbohydrate and calcium-ion dependent [38]. In another example, the p24 protein Emp24p is required for efficient ER-export of specific secretory cargo in yeast, including the GPI-anchored protein Gas1p [42]. Additional experiments have shown that Emp24p forms direct crosslinked products with Gas1p in ER-derived vesicles [39]. Finally, a recent report indicates that Erv29p acts as an export receptor for another subset of soluble secretory proteins in yeast. Erv29p was identified as an abundant integral membrane protein on ER-derived vesicles and is predicted to contain multiple membrane-spanning domains [43]. In cells lacking Erv29p, the soluble cargo proteins glyco-pro-α-factor, carboxypeptidase Y and proteinase A are inefficiently exported from the ER, whereas other soluble and transmembrane cargoes exit the ER at normal rates [40,44]. *In vitro* studies show that the glyco-pro-α-factor cargo protein requires Erv29p for packaging into COPII vesicles, and this cargo protein is detected in complexes with Erv29p in budded vesicles. As expected for a receptor-mediated process, export of glyco-pro-α-factor from the ER is saturable upon overexpression of this cargo protein. Furthermore, elevating the expression level of Erv29p increases the capacity for glyco-pro-α-factor export from the ER [40].

ERGIC53, p24 proteins and Erv29p are proposed to recognize and bind to specific export signals contained

within distinct soluble cargo molecules. Presumably, binding is regulated such that fully folded secretory proteins are bound by the receptor in the ER and then released in post-ER compartments. Possible changes in lumenal pH and/or Ca^{2+} concentration within distinct membrane compartments could regulate receptor–cargo interactions. Alternatively, COPII-dependent oligomerization of membrane receptors could influence receptor–cargo interactions. Regardless, further refinement of specific export signals in soluble cargo molecules [45,46] and an elucidation of mechanisms that govern receptor–cargo interactions remain key tests of the receptor-mediated export model.

Concluding remarks

Specific amino acid sequence motifs in transmembrane cargo molecules have been identified that are required for concentrative sorting into ER-derived vesicles. Both the di-acidic and di-hydrophobic motifs in export cargo bind to subunits of the COPII coat, providing a direct mechanism for cargo selection. The Sec23–Sec24 protein complex serves a role in cargo recognition, whereas the Sar1 GTPase appears to regulate interactions between coat and cargo. Structural studies on coat–cargo complexes are now needed to provide a molecular view of cargo recognition. Growing evidence suggests that certain soluble secretory proteins exit the ER in a receptor-mediated process; however, other types of cargo might depart in a bulk-flow manner. The identification of transplantable export signals contained within soluble cargo proteins will be a crucial step to explore underlying molecular mechanisms.

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