Published in final edited form as:

Biochim Biophys Acta. 2012 August ; 1821(8): 1040–1049. doi:10.1016/j.bbailip.2012.01.005.

Vesicle-mediated ER export of proteins and lipids

Amanda D. Gillon⁺, Catherine F. Latham⁺, and Elizabeth A. Miller^{*}

Department of Biological Sciences, Columbia University, New York, NY USA

Abstract

In eukaryotic cells, the endoplasmic reticulum (ER) is a major site of synthesis of both lipids and proteins, many of which must be transported to other organelles. The COPII coat – comprised of Sar1, Sec23/24, Sec13/31 – generates transport vesicles that mediate the bulk of protein/lipid export from the ER. The coat exhibits remarkable flexibility in its ability to specifically select and accommodate a large number of cargoes with diverse properties. In this review, we discuss the fundamentals of COPII vesicle production and describe recent advances that further our understanding of just how flexible COPII cargo recruitment and vesicle formation may be. Large or bulky cargo molecules (eg. collagen rods and lipoprotein particles) exceed the canonical size for COPII vesicles and seem to rely on the additional action of recently identified accessory molecules. Although the bulk of research has focused on the fate of protein cargo, the mechanisms and regulation of lipid transport is equally critical to cellular survival. From their site of synthesis in the ER, phospholipids, sphingolipids and sterols exit the ER, either accompanying cargo in vesicles or directly across the cytoplasm shielded by lipid transfer proteins. Finally, we highlight the current challenges to the field in addressing the physiological regulation of COPII vesicle production and the molecular details of how diverse cargoes, both proteins and lipids, are accommodated.

Keywords

COPII; cargo export; collagen; lipids; vesicle; endoplasmic reticulum

1. Introduction: the COPII coat marks the way out

The ER is a remarkably productive organelle, being the site of secretory protein synthesis and a major source of intracellular lipid synthesis. Furthermore, it maintains a highly dynamic structure, comprising the nuclear envelope, membrane sheets, tubules and cisternae that are fully interconnected [1–3]. To maintain this structure and functionality requires tight regulation of a number of processes including protein and lipid transport. Within the spectrum of diverse eukaryotic cells, the proportion of individual subcompartments, their intracellular distribution and their specialized functions may vary, however the global morphology of the ER is well conserved as are the methods by which secretory cargo and lipids are managed for efficient intracellular delivery to downstream compartments. Central to this process is the generation of ER-derived transport vesicles, known as COPII vesicles,

© 2011 Elsevier B.V. All rights reserved.

^{*}Corresponding author: Address: 1212 Amsterdam Ave., MC2456, New York, NY 10027, em2282@columbia.edu, Phone: +1 212-854-2264, Fax: +1 212-865-8246.

⁺these authors contributed equally

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

named for the protein coat that shapes them [4, 5]. Originally characterized in yeast cells, these highly conserved proteins also function in metazoans, serving as the main conduit of protein traffic out of the ER. In humans, defects in ER export have serious consequences, leading to developmental disorders and disease such as the autosomal-recessive disease cranio-lenticulo-sutural dysplasia (CLSD), congenital dyserythropoietic anemia, and fat-malabsorption diseases, chylomicron retention disease and Anderson disease [6–9].

1.1 COPII coat assembly

The COPII coat comprises five proteins that are recruited to the ER membrane to induce membrane curvature and mediate specific capture of cargo proteins (Figure 1). COPII vesicle biogenesis is initiated by the activation of a small GTP-binding protein, Sar1, through its guanine nucleotide exchange factor (GEF), Sec12 [10]. Upon exchange of GDP for GTP, Sar1 exposes an amphipathic α -helix that anchors the protein to the ER membrane, initiating membrane deformation and recruitment of additional coat components [11–13]. Sar1-GTP then stimulates the recruitment of the “inner” coat, Sec23/24, which is responsible for capturing cargo proteins into the nascent vesicles. Structural studies of the Sec23/24 complex revealed a concave surface with curvature that matches that of a 60 nm vesicle, implicating the inner coat in assisting membrane bending, perhaps by capturing curvature initiated by Sar1 [12]. The Sec13/31 heterotetramer, the “outer” coat, is subsequently recruited to stabilize the cargo-bound “pre-budding” complexes and likely completes the vesicle budding process by promoting further membrane curvature and fission [4, 14, 15]. The GTPase activity of Sar1 and its N-terminal α -helix are also important for membrane scission associated with vesicle release, although the mechanism remains unclear [13, 16]. These five coat proteins, Sar1, Sec23, Sec24, Sec13 and Sec31, are the minimal machinery required for vesicle formation, being sufficient to drive release of coated vesicles *in vitro* from synthetic liposomes [17].

1.2 Regulation of COPII coat function

At the heart of COPII function is the GTP cycle of the coat: GTP-loading onto Sar1 drives assembly, and coat shedding following vesicle release likely proceeds because GTP hydrolysis leads to Sar1 release and further coat destabilization. It has been known for some time that Sec23 is the GTPase-activating protein (GAP) for Sar1 [18], and the crystal structure of Sec23 complexed with the Sar1 revealed that Sec23 inserts a key catalytic arginine residue (Arg 722) into the active site of Sar1 [19]. This so-called “arginine finger” is a common structural feature of GAP stimulation of Ras-family GTPases and functions by stabilizing the transition state [20]. Like other small G-proteins, Sar1 has relatively poor intrinsic GTPase activity and efficient hydrolysis requires assistance not just from its GAP, Sec23, but also from the outer coat protein, Sec31. This phenomenon was first observed using synthetic liposomes to monitor coat dynamics in real-time [21]. Recruitment of Sec13/31 to the pre-assembled Sar1-GTP-Sec23/24 complex stimulated the GTPase activity of Sar1-Sec23 ~ 10-fold and induced rapid disassembly of the coat [21]. In more recent studies, a 40-residue active fragment of Sec31 was defined; structural analysis revealed that this lies across the membrane-distal face of the Sec23/Sar1 complex, and stimulates the Sec23 GAP activity by optimally positioning the catalytic residues [19].

That the full GTPase activity of the COPII coat is realized by its own assembly has created an interesting paradox: if coat assembly triggers disassembly, how is coat stability prolonged to permit vesicle production? Additional levels of regulation must influence either the GTP cycle or coat stability to permit productive vesicle formation. In part, the answer to this conundrum may come from Sec12, which can extend the half-life of the coat on synthetic liposomes from ~2 sec. to ~15 min., suggesting Sec12 action trumps the GAP activity of Sec23 and favors maintenance of the COPII coat even in the presence of GTP [22, 23].

Furthermore, although they are not essential for vesicle biogenesis, cargo molecules may also influence COPII coat dynamics [17]. Simultaneous real-time analysis of coat recruitment and the GTP cycle revealed that Sec23/24 remained associated with liposomes that contained cargo while Sar1 underwent multiple rounds of GTP hydrolysis [23]. This observation is supported by in vivo analyses of coat turnover at ER exit sites (ERES): Sec23/24 has a notably longer half-life than Sar1, but this difference was greatly diminished when cargo was depleted due to an accelerated turnover of Sec23/24 [24]. Thus it appears that the presence of cargo provides additional affinity that enables Sec23/24 to remain membrane-associated even after Sar1 has hydrolyzed GTP. These new measurements support older findings that Sec23/24 and Sec13/31 remain on vesicles generated in vitro from yeast ER membranes even after Sar1 has hydrolyzed GTP and been released [4]. Indeed, the persistence of the coat on COPII vesicles may play a key role in ensuring the directionality of vesicle traffic: vesicle-bound Sec23 interacts with the tethering complex, TRAPP, in a manner that is regulated by a Golgi-bound kinase suggesting that the coat marks vesicles destined to fuse with the Golgi and that coat release may be further triggered by phosphorylation events [25, 26]. Cargo may also influence coat assembly by stimulating recruitment of Sec13/31; single-molecule microscopy on an artificial planar lipid bilayer revealed that cargo-bound Sar1-Sec23/24 partially dimerizes prior to Sec13/31 recruitment [27]. Tabata and colleagues proposed that the cargo-containing prebudding complex (Sar1-Sec23/24-cargo) provides the ideal scaffold for Sec13/31 recruitment that subsequently leads to productive vesicle budding. Further candidates for coat regulation on the ER membrane include p125 [28], ALG-2 [29], Sec16, and TRK-fused gene, TFG-1 [30, 31].

1.3 Organization of ER exit sites

Another layer of potential regulation of ER export revolves around the clear sub-division of the ER into discrete domains. COPII vesicles appear to arise from distinct regions of the ER known as transitional ER (tER) or ERES. These structures, marked by COPII proteins, are relatively stable, largely immobile structures of approximately 0.5 μm that form at small ribosome-free regions within the rough ER [14, 32–35]. In mammalian cells, ERES are not evenly distributed along the ER membrane but are found in discrete sites that face towards small structures, known as vesicular tubular clusters (VTCs) or the ER-Golgi intermediate compartment (ERGIC) [32]. The budding yeast, *Pichia pastoris*, similarly has discrete ERES that are relatively few in number and are apposed to the Golgi, whereas *Saccharomyces cerevisiae* appears to lack this higher layer of organization with COPII vesicles budding across the entire ER membrane [36]. Two proposed functions of ERES are (i) to enable cargo destined for export to be efficiently packaged and (ii) to ensure that ER resident proteins and other non-cargo substrates remain in the ER.

The molecular makeup and structure of ERES is an emerging area that seeks to expand our understanding of the mechanisms by which unique subdomains are maintained in such a fluid membrane environment. The lipid composition of the membrane has long been proposed to play a role in ER trafficking, however only relatively recently has concrete evidence emerged that suggests phosphatidylinositol (4)-phosphate (PtdIns4P) is specifically enriched in ER subdomains, potentially acting to regulate the recruitment of Sar1 [37]. ERES themselves may also come in distinct flavors: Castillon and colleagues identified that yeast maintain three distinct ERES populations that differ by cargo they concentrate for export. The three different populations carry either soluble cargoes like pro- α -factor, transmembrane cargoes like amino acid permeases, or GPI-anchored proteins [38]. Such segregation of ERES is consistent with previous findings that suggest there also at least two distinct COPII vesicle populations incorporating either GPI-anchored proteins or non-GPI-anchored proteins [39]. How these different identities are established and maintained is unclear, but the requirement of different lipids in the ERES as well as the recruitment of

specific COPII components (Sec24, Lst1, Iss1) [40] could also define specific characteristics for distinct ERES.

The only known player in ERES organization is the relatively large (~240 kDa) peripheral membrane protein, Sec16. First identified in a genetic screen in *S. cerevisiae* as a secretion mutant, this essential protein is conserved across species, marks ERES and could provide a scaffold to support coat assembly through its interactions with each of the COPII coat proteins [30, 41–43]. Although the precise molecular function of Sec16 has not been established, the current view is that it is recruited to ERES upstream of the COPII subunits and is required for maintenance of these structures. Depletion of Sec16 from mammalian cells disperses ERES on the membrane [35]. Regulation of Sec16 and these early secretory pathway steps were recently reported to be, in part, controlled by two kinases, the Mitotic-Associated Protein Kinase (MAPK) Extracellularly regulated kinases (ERKs) ERK2 and ERK7 [44]. ERK2 directly phosphorylates human Sec16 at threonine 415 resulting in the recruitment of Sec16 to ERES, leading to up-regulation of ERES and in turn, up-regulation of ER-to-Golgi transport [44]. Conversely, upon nutrient starvation, ERK7 induces Sec16 phosphorylation releasing Sec16 from the tER sites, ERES disassembly and hence diminished ER-to-Golgi transport [45].

2. ER export of protein cargo

The process of accurate and selective recruitment of cargo proteins into nascent COPII vesicles is an integral part of the fidelity of ER export and transport through the secretory pathway. Indeed, the sheer volume and diversity of molecules that traffic through the ER is testament to the flexibility of this process: it is estimated up to one-third of all proteins in yeast, ~70% of hepatocyte proteins and ~6000 proteins in human cells traffic through the ER for secretion or delivery to other organelles of the endomembrane system [46, 47]. Upon translation/translocation into the ER, chaperones and folding enzymes recognize the newly synthesized proteins to complete protein assembly prior to ER egress via concentrated, signal-mediated export or bulk-flow [48]. To ensure the effective onward transport of correctly folded proteins and the removal of aberrant proteins, protein biogenesis and trafficking is highly regulated [49], and is intertwined with the COPII functionality, to ensure that only acceptable cargo are released from the quality control system of the ER.

2.1 Concentrative, signal-mediated ER export

In general, selective export from the ER relies on interaction (either direct or indirect) between an export signal on the cargo protein and a cargo-recognition site on the Sec24 component of the COPII coat [50, 51]. Export signals enable proteins to be concentrated 3- to 50-fold [52] in COPII vesicles over their prevailing concentration in the ER. Unfortunately, these signals do not fall into a single consensus sequence. Given the diversity of cargo clients, perhaps this diversity in export motifs is not surprising, especially considering that Sec24 appears to function as a multi-valent adaptor platform capable of binding multiple independent signals (see section 2.1.2 below).

2.1.1 ER export signals—Although specific export signals for the vast majority of secretory proteins remain unknown, a number of motifs found on transmembrane cargoes that bind to Sec24 have been defined, including di-acidic, hydrophobic and aromatic motifs [53–55] (Figure 2). The cytoplasmically exposed di-acidic motif (D/E-X-D/E) is conserved among diverse proteins from various organisms, including the vesicular stomatitis virus glycoprotein (VSV-G), mammalian transporters for serotonin (SERT), and yeast Sys1p and Gap1 [54, 56, 57]. Interestingly, the yeast proteins Gap1 and Sys1 both use superficially similar di-acidic signals (D-I-D and D-x-E, respectively), yet these different proteins seem to bind to different sites on Sec24, and are thus sorted independently [50]. Another well-

defined signal is the di-hydrophobic motif (FF, YY, LL or FY) located on the type I membrane protein ERGIC-53 where the di-phenylalanine (FF) ER export signal can be functionally substituted with di-tyrosine (YY), di-leucine (LL) and other hydrophobic residues [55, 58].

Although most transmembrane protein cargoes can in principle interact directly with Sec24 through motifs in their cytosolic domains, soluble proteins present in the ER lumen and some membrane-bound proteins require cargo-receptors to bridge the membrane and make contact with the COPII coat (Figure 2). Cargo receptors are typically transmembrane proteins that cycle between the ER and Golgi. For example, ERGIC-53 is a protein with a single transmembrane domain that possesses a di-phenylalanine motif for forward COPII transport and a di-lysine motif for retrograde COPI transport [47, 53, 59]. Multiple soluble cargoes engage ERGIC-53 including cathepsin C, cathepsin X and blood clotting factors V and VIII [60]. ERGIC-53 mutants result in blood clotting disorders, presumably as a result of defects in ER egress of ERGIC-53 client cargoes [60]. ERGIC-53 interacts directly with Sec23/Sec24 complex and upon reaching the ERGIC, the cargo is released and ERGIC-53 is recycled back to the ER [59, 61]. Furthermore, a recently identified putative cargo receptor, TANGO1, is of particular interest due to its involvement with a large/bulky cargo, collagen VII [62] and is further discussed below in section 2.2.

2.1.2 Sec24-mediated capture of cargo—Biochemical, structural and genetic studies of yeast Sec24 pioneered our understanding of cargo selection by this COPII component and elucidated three independent cargo-binding sites, now defined as the A-, B- and C-sites [50, 51] (Figure 2). Each cargo-binding site recognizes distinct ER export motifs and appears to act independently of the others. The A-site mediates interaction with the YxxxNPF motif on Sed5p through a hydrophobic pocket [51]. The B-site, a groove on the opposite side of Sec24 to the A-site, recognizes the three motifs; Dx_E, LxxME and LxxLE that belong to Sys1, Sed5 and Bet1 respectively, and the C-site interacts with the soluble N-ethylmaleimide attachment receptor (SNARE) protein, Sec22, via a conformational epitope [12, 50, 51]. Interestingly, Bet1, Sys and Sed5 utilize subtly distinct binding modes despite binding the same site, suggesting the B-site can accommodate a diversity of proteins [51]. Furthermore, although the B- and C-sites are immediately adjacent to each other, they are not allosterically linked and appear not to cooperate in the capture of cargo proteins [63]. Cross-talk between different binding sites has not been seen, although the possibility remains that some allosteric effects may occur, despite the fact that the crystal structures of Sec24 bound to different cargoes are remarkably similar.

In mammalian cells, an additional site on Sec24 has been characterized structurally that selectively binds membrin and syntaxin5 via an I-X-M motif that is highly conserved, both in sequence and position, in higher eukaryotes [64]. This common binding site is on the equatorial surface of Sec24, with only the isoleucine and methionine side chains of the I-X-M motif making substantial contacts with Sec24 [64]. Moreover, the transporter for serotonin (SERT) was the focal point of a recent study on Sec24-mediated export from ER. Mutagenesis, mass spectrometry and siRNA analysis adeptly characterized a Sec24-binding motif (SERT-⁶⁰⁷RI⁶⁰⁸) and the corresponding cargo-binding motif (Sec24-⁷⁹⁶DD⁷⁹⁶) [56]. It seems entirely likely that additional sites of interaction remain to be identified on the ample surface of Sec24, and understanding how binding at these different sites might be regulated (or cross-regulated) remains an ongoing challenge.

2.1.3 Increasing the diversity of cargo capture—One mechanism for coping with the diversity of cargo during ER export is to amplify the cargo binding capacity of Sec24. This is driven in part by the multiple independent binding sites on Sec24, but also stems from duplication of Sec24 function via its paralogs. Yeast has three Sec24-related proteins: Sec24

itself, Iss1/Sfb2 (56% identical to Sec24) and Lst1/Sfb3 (23% identical to Sec24). Humans have four Sec24 isoforms that appear to fall into two subclasses: Sec24A and Sec24B share 75% sequence identity; Sec24C and Sec24D share 66% identity; conversely, Sec24A and Sec24C share only 31% identity. These relationships are reflected in the distinct cargo binding selectivity exhibited by the different isoforms; Sec24A/B selectively interact with the SNARE Sec22 and Sec24C/D recognize the conserved I-X-M sequence on membrin and syntaxin as mentioned above (section 2.1.2) [64]. Recently, Merte and colleagues (2009) demonstrated that Sec24B binds specifically to Vangl2, an essential membrane protein involved in the Wnt signaling pathway that establishes planar cell polarity. Dysfunction of this pathway in mice, including by mutation of Sec24B, results in neural tube defects that present as disorders including spina bifida (failure of caudal tube closure), anencephaly (failure of rostral neural tube closure) and craniorachischisis [65, 66]. In yeast, the functions of Sec24 paralogs remain to be fully explored. Knockout of the essential *SEC24* gene in yeast cells can be partially rescued by overexpression of the non-essential Iss1, but not Lst1, suggesting some functional redundancy [40, 67]. Conversely, Lst1, also non-essential, is required for efficient transport of the GPI-anchored protein Gas1 and the plasma membrane H⁺-ATPase, Pma1 [40], but alone is unable to support secretion in vivo. The apparent requirement for Sec24, and to a lesser extent Iss1, has been attributed to its ability to bind the SNARE protein, Bet1, which is required for vesicle fusion with the Golgi apparatus and hence, for viability [68]. The inability of Lst1 to package SNAREs or support viability in vivo indicates that COPII vesicles likely employ a combination of Sec24 and its paralogs [69]. Although Lst1 is not essential, it is interesting to note that a combination of Sec23/Lst1 and Sec23/24 generated vesicles that were larger than vesicles with Sec23/Sec24 alone [69]. The reason for this remains unknown but may be due to differences in the structure of Lst1, or more indirectly caused by the unique properties of the Lst1-specific cargoes [70]. Such cargo proteins include the plasma membrane H⁺-ATPase Pma1, which is known to form large oligomeric complexes, and the p24 proteins that function as cargo adaptors for GPI-anchored proteins, which in turn have specific lipid-domain requirements for efficient ER export [38, 70] (see section 2.4).

2.1.4 Export of GPI-anchored proteins—Glycophosphosphatidylinositol-anchored proteins (GPI-APs) are a specialized class of proteins located primarily on the extracellular face of the plasma membrane (PM), tethered by a lipid-glycan anchor rather than by protein transmembrane domains. Trafficking of GPI-APs from the ER to the PM seems dependent on their lipid as well as protein portions. GPI-AP are initially synthesized as single-pass transmembrane proteins, and post-translationally modified in the ER where the transmembrane domain is proteolytically removed and the GPI anchor is attached and remodeled [71]. GPI anchor remodeling is required for efficient recruitment to ERES and subsequent transport to the PM via the secretory pathway [72]. GPI-APs are thought to be organized into membrane microdomains that are defined by localized membrane clusters enriched in certain proteins as well as sterols and sphingolipids [73, 74](Figure 2). Trafficking of GPI-APs is dependent on the presence of both sterols [75] and sphingolipids, especially ceramides [76, 77] that could stabilize the anchor within the lipid bilayer during secretion. Several studies have even suggested that GPI-APs are segregated into separate ERES from non-GPI-anchored cargo [38, 39] and that the membrane bilayer at these sites could also be enriched in these lipids.

Within the ER, GPI-APs are localized exclusively to the luminal leaflet of the ER membrane, physically separated from the COPII coat. Consequently, as with a soluble luminal cargo, a cargo receptor that spans the ER membrane and interfaces with COPII proteins is required for recruitment into transport vesicles. The cargo receptors implicated in transport of GPI-APs in both yeast and mammalian cells are the p24 proteins [78–82], a conserved family of proteins that have a large luminal domain, a transmembrane domain

and a short cytosolic tail that binds to COPII proteins [84]. The current view is that four p24 proteins – Emp24p, Erv25p, Erp1p and Erp2p in yeast – form a heterooligomeric complex that mediates export of GPI-APs via incorporation into COPII vesicles [85, 86]. Genetic analyses suggest that enzymes involved in GPI-anchor remodeling could act together with p24 proteins for efficient trafficking of GPI-APs [87]. Interestingly, both the GPI-AP, Gas1, and the p24 complex are packaged into vesicles by Sec24 isoform, Lst1 [40] (Figure 2). These findings raise an interesting question: how does the p24 complex coordinate recruitment and transport of all GPI-APs, a heterogeneous group of proteins with diverse structures and functions? One explanation is that recognition of GPI-APs could rely largely on the lipid-glycan anchor rather than the protein itself [88]. This hypothesis is supported by recent studies showing that p24 proteins associate only with GPI-APs that contain correctly remodeled GPI-anchors and that this association is essential for successful trafficking to the Golgi [89, 90]. Exactly how the p24 complex organizes GPI-APs into ERES and whether these ERES are distinct from other COPII-dependent protein cargo is yet to be determined.

2.2 Transport of large and bulky cargo molecules

Since COPII transport carriers have classically been described as spherical vesicles with a diameter of ~60–100 nm, a major challenge in the field is understanding how large/bulky proteins are able to be exported from the ER. In vitro, purified Sec23/24 and Sec13/31 are able to generate empty COPII cages up to 100 nm [91, 92], but these remain too small to accommodate large/bulky cargo proteins that can range from 300 to 1000 nm in diameter. Collagens are one example of a very large cargo, arranging into 300–400 nm rod-like structures in the ER. COPII proteins have only relatively recently been implicated in the transport of procollagen [93], supported by the identification of a missense mutation in *SEC23A* (F382L) that leads to the autosomal-recessive disease craniolenticulosutural dysplasia (CLSD) a disease that causes facial dysmorphisms, skeletal defects and sutural cataracts [94] as a result of impaired collagen export from the ER [6, 15, 19].

The question of how such extraordinary cargoes are transported has freshly intensified. A genome-wide screen using siRNA in *Drosophila* tissue culture cells revealed two proteins, Transport and Golgi Organization 1 (TANGO1) and cutaneous T-cell lymphoma-associated antigen 5 (cTAGE5), that concentrate as a dimer at ERES and are required for collagen VII secretion [62, 94]. TANGO1 binds to Collagen VII through its SH3 domain in the ER lumen and to Sec23/24 via its proline rich domain (PRD) in the cytoplasm [62, 95] (Figure 1). Since both TANGO1 and Sec31 contain a PRD that interacts with the COPII inner layer, Sec23/24, TANGO1 could influence Sec13/31 recruitment and thereby stall coat assembly, and/or Sar1-GTP hydrolysis that causes vesicle scission [13, 16]. The CLSD-associated mutation, F382L, maps close to the binding site of Sec31 [19] resulting in poor recruitment of Sec13/31 that likely inhibits vesicle formation [15]. Temporarily halting vesicle scission would allow for propagation of the coat over an extended area, allowing the vesicle to fully enclose the large cargo. Upon incorporation of collagen into a “mega vesicle”, the TANGO1 PRD would dissociate from Sec23/24 permitting Sec13/31 to be recruited and complete vesicle release [62, 96]. Neither TANGO1 nor cTAGE5 are incorporated into the budded vesicles and have thus been dubbed a “kinetic timer” for mega vesicle biogenesis rather than a classical cargo adaptor [96].

As discussed later in this review (see section 3.2 below), lipoproteins such as chylomicrons are another class of large cargoes (75–450 nm) that rely on COPII coat proteins as well as other protein players for successful ER export.

2.3 Non-selective ER export and unconventional modes of secretion

Non-selective export of soluble and membrane proteins, also termed passive or “bulk flow”, is a route whereby proteins are transported from the ER at their prevailing ER concentrations, being packaged stochastically as the membrane and small luminal volumes are engulfed in a COPII vesicle [97]. Although not a highly efficient mode of transport, bulk flow is still utilized by select proteins including two abundant secretory proteins, amylase and chymotrypsinogen from mammalian pancreatic exocrine cells [3]. Electron microscopy analysis resolved the presence of chymotrypsinogen and amylase in COPII vesicles at concentrations similar to the ER lumen unlike other specific secretory cargoes, which are concentrated at ERES [3]. Chymotrypsinogen and amylase were also visualized in tubular structures purportedly en route to the Golgi compartment. More recently, the importance of bulk flow was more quantitatively evaluated in mammalian cells utilizing a rapidly folding domain of a viral capsid protein, predicted to not interact with typical folding machinery [98]. Bulk flow of this “inert” protein was surprisingly rapid, and still dependent on COPII vesicle formation, even in the absence of specific export signals [98]. That bulk flow can result in rapid and efficient secretion should lead to a deeper re-evaluation of the extent to which this phenomenon may apply to endogenous secretory proteins, although it is unlikely that such substrates will be similarly unencumbered by interactions with cellular folding and post-translational modification machinery.

Another mode of clearing proteins from the ER is via a specialized form of autophagy, whereby misfolded proteins bypass the canonical secretory pathway and are delivered directly to the vacuole/lysosome [99, 100]. This pathway is likely required to efficiently rid the ER of large protein aggregates that are either immobile or otherwise unable to enter into standard COPII vesicles. During this specialized autophagy, ER membranes are thought to be selectively sequestered for the generation of autophagosomes, double-membrane bound vesicles. This process is activated by the unfolded protein response (UPR), a physiological pathway induced by accumulation of misfolded proteins in the ER, and is thought to regulate the size of the ER, which becomes enlarged upon UPR induction [100]. Intriguingly, the ER is also implicated in more canonical autophagy pathways, for which the source of membrane remains poorly understood. Recently, a well-characterized player in ER-to-Golgi transport, the small GTPase, Rab1 (Ypt1 in yeast), and its GEF, transport protein particle (TRAPP) III, were shown to be required for autophagy [99]. These regulators of secretion may moonlight as regulators of vesicle tethering and fusion in generating autophagosomal membranes, although the root source of the donor membranes remains unclear.

In addition to these diverse COPII-dependent and COPII-independent pathways, a non-conventional protein secretion mechanism has been proposed for proteins that contain signal peptides (and thus enter the ER), but are not dependent on COPII vesicles to exit the ER [101]. The best-characterized example of a protein that takes this route is the yeast secretory glycoprotein, Hsp150, which contains a signal peptide but is released to the cell surface even in *sec24* or *sec13* mutant cells [102–104]. However, the mechanism used and the number of cargo proteins that might employ this pathway are not known.

3. Lipid traffic from the ER

Although primarily studied for its role in the synthesis of secretory proteins, the ER is also central to endomembrane homeostasis as a key site of lipid synthesis. Over 1000 different lipid species comprise cellular membranes [105], forming three major groups based on their chemical structures – glycerophospholipids, sterols and sphingolipids. The distribution of these lipids varies within the cell, with different intracellular compartments enclosed by membranes with distinct compositions with respect to each of these three lipid classes. Most cellular bilayers are composed primarily of glycerophospholipids such as

phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine, with the proportion of sphingolipids and sterols varying greatly. Eukaryotic plasma membranes are relatively rich in sphingolipids and sterols, comprising up to 30% [106], whereas the ER characteristically has much lower levels of both classes of these lipids, closer to 5% [107]. Furthermore, even within the membrane of a given organelle, certain lipids can be heterogeneously distributed, which could be driven in part by interactions with embedded membrane proteins (reviewed in [108, 109]). Since most lipid synthesis occurs or originates in the ER, this intracellular lipid heterogeneity is largely the result of lipid sorting events that occur as membrane flows through the secretory pathway, supplemented with non-vesicular direct lipid transfer between compartments mediated by lipid-transfer proteins. Furthermore, the ER serves as a site of cellular response to and regulation of dietary lipids; notably, sphingolipid and sterol levels are tightly regulated via control over synthesis that occurs primarily in the ER, although only low levels of both lipid classes are present in the ER membrane itself [110]. Dietary lipids absorbed by cells in the small intestine and liver also make their way to the ER where they are packaged into lipid particles or bodies for transport via the bloodstream to storage sites in the body such as adipose tissue. Thus, both dietary lipids absorbed by the cell and lipids synthesized in the ER must be transported through the cell to fulfill the requirements of the downstream intracellular compartments.

3.1 Vesicular transport of lipids from the ER

Since COPII-coated vesicles are sculpted from the ER membrane, they are an important source of ER egress of bulk lipids. Indeed, the lipid cargo of a COPII vesicle may be equally important as the secretory protein components, being the source of membrane lipids for downstream organelles. Yet the specificity of this process remains poorly studied. Non-specific bulk-flow is likely to be the primary mechanism for incorporating glycerophospholipids into COPII vesicles since this is the major lipid class forming the ER membrane bilayer. Sterols and sphingolipids, however, are depleted from the ER membrane and become progressively enriched in later organelles along the secretory pathway. Transport of complex sphingolipids such as sphingomyelin and glycosphingolipids is heavily dependent on vesicular transport [111], yet how these lipids might be preferentially exported from the ER in vesicles remains unclear. The favored model suggests that lipid segregation within the membrane, driven by lipid-lipid interactions, guides preferential incorporation of certain lipids into vesicles. For example, sphingolipids segregate from phosphatidylcholine into discrete membrane microdomains in all compartments along the secretory pathway [112]. GPI-APs, which rely on the Sec24 isoform, Lst1p, for cargo selection into COPII vesicles, are concentrated in these membrane microdomains (see Fig. 2B) and could contribute to protein sorting by forming distinct ERES for COPII transport. Sterols also segregate from other lipids in the bilayer by associating with protein transmembrane domains of specific lengths [113], which could also contribute to protein sorting. Intracellular transport of sterols, unlike sphingolipids however, is only minimally affected by blocking vesicular traffic [114], so it must rely on an alternative pathway. Furthermore, dietary lipid particles generated in the ER are too large to be accommodated in a canonical COPII vesicle even though the COPII coat is required for their secretion. Clearly, additional mechanisms of intracellular lipid transport are necessary to traffic newly-synthesized sterols and sphingolipids to other organelles and to secrete dietary lipoproteins into the bloodstream.

3.2 Vesicular transport of large lipoprotein cargo

Cholesterol and fatty acids (FAs) absorbed by the cell are sequestered in the ER and subsequently packaged into a variety of lipoprotein particles, including chylomicrons in cells of the small intestine and very low-density lipoproteins (VLDLs) in the liver. These particles enable highly hydrophobic lipids, such as cholesterol, to traverse the aqueous

environment of the cytoplasm during secretion. Incoming FAs are most likely escorted to the ER by fatty acid binding proteins (FABPs) and can also be acylated or converted to triacylglycerol in the ER where they contribute to composition and biogenesis of lipoprotein particles [115]. During fasting, VLDLs are released into the bloodstream from the liver to provide adipose, cardiac, and skeletal muscle for energy production or storage, whereas after food intake, chylomicrons are generated from dietary fats and secreted into the bloodstream to be absorbed by other tissues in the body. Lipoproteins contain a low-density core of neutral lipids protected from the aqueous environment by an external layer of phospholipids and proteins, principally the highly-hydrophobic family of apolipoprotein B (ApoB) proteins. Apolipoprotein B100 (ApoB100) is the major ApoB protein component of VLDLs in the liver [115], and a truncated form, apolipoprotein B48 (ApoB48), is the only ApoB protein in chylomicrons [116].

These large lipid particles form in the ER and exit via the secretory pathway, followed by maturation in the Golgi [117] (Figure 1). At ~250 nm, immature chylomicrons and VLDL particles within the ER are much larger than the ~100 nm of the largest observed COPII vesicle cage [91] although dependence on COPII proteins for transport has been reported for both species of lipid particles [7, 115]. The COPII GTPase, Sar1B, has been implicated in secretion of these particles by genetic analysis of lipid storage diseases involving intracellular chylomicron retention [7] and the COPII cargo selection subunit, Sec24C (and to some extent Sec23) seems to be required for targeting prechylomicron transport vesicles (PCTVs) to the Golgi [119]. Vesicles carrying lipid particles, however, are not generated exclusively by the COPII machinery. Recent studies have identified large specialized vesicles that export chylomicron precursors, prechylomicrons, and VLDLs from the ER with protein markers distinct from canonical COPII vesicles [118, 120]. These special vesicles, the PCTVs, are generated by a specific budding complex, comprised of vesicle-associated membrane protein 7 (VAMP7), ApoB48, liver FABP (L-FABP) and CD36 as well as COPII proteins [120]. L-FABP was shown to be competent for forming PCTVs without the COPII coat, although these vesicles were not competent for Golgi fusion [121]. Less is known about VLDL transport vesicles (VTVs), which were identified more recently than PCTVs and contain ApoB100, as well as the COPII GTPase, Sar1 and SNARE protein, Sec22b [118] which is required for fusion with the Golgi [122].

3.4 Non-vesicular lipid transport

In addition to export from the ER via COPII-coated vesicles or larger lipoprotein particles, evidence for non-vesicular mechanisms for lipid transport is well documented. Lipid redistribution within the cell still occurs under conditions where vesicular transport has been blocked by drug treatment [123, 124]. It is also evident in organelles such as lipid droplets and mitochondria that are not part of the secretory pathway [125–128]. Intracellular movement of lipids can also occur at membrane contact sites (MCS), which are small cytosolic gaps of 10–20 nm between the closely apposed membranes of different organelles.

MCSs have been observed between the ER and most other organelles including the PM, Golgi, lipid droplets and mitochondria. Transport of Ca^{2+} , metabolites and lipids can occur at MCSs, which are stable entities enriched in proteins such as enzymes for lipid synthesis and transport or inter-organelle transport channels [129]. Traversing the short distance across the cytosol at MCS junctions occurs either spontaneously, by slow release and diffusion of monomeric lipids through the cytosol. Alternatively, lipids can be carried across MCSs by proteins such as a lipid transfer proteins (LTPs). LTPs are thought to extract specific monomeric lipids from a donor membrane and shield them in a lipid-binding pocket before unloading into the membrane of the acceptor organelle. Many LTPs have been studied *in vivo* and characterized structurally, including CERT, for ceramide transport from the ER to the Golgi [130] and members of the steroidogenic acute regulatory (StAR) protein

family that have varying lipid specificities [131]. Studies in both mammalian and yeast cells have shown that sterols synthesized in the ER are transported to other cellular membranes, such as the PM, almost exclusively by non-vesicular transport mechanisms [114, 132, 133]. In fact, all three classes of lipids can be transported by LTPs and transfer often occurs at MCSs, trading lipids between the ER and other organelles as they are required.

4. Conclusions and perspectives

New challenges are now emerging in understanding export from the ER. The minimal components of canonical COPII vesicle formation have been characterized extensively, and recent efforts are focusing on not only the flexibility of the COPII machinery but also how this machinery is coordinated in vivo. While recapitulating the minimal COPII machinery in vitro can describe the molecular detail of vesicle formation, it cannot report on any diversity in the vesicles formed in the cell. For example, the presence of distinct ERES in vivo, where specific cargoes are segregated from others [38], raises the question of how the vesicles generated from these sites vary in their protein and lipid composition and how this segregation is achieved. Perhaps a specific combination of COPII isoforms is required for cargo recruitment or engaging specific cargo-dependent regulatory mechanisms for their transport. To begin to address this question, one approach could be to isolate distinct species of COPII vesicles using cargo proteins as markers and determine their complete protein and lipid complement, similar to the detailed characterization of synaptic vesicles [134]. Vesicles isolated using protein markers observed in separate ERES could identify any differences in the coat or identify novel molecules involved in regulating export of specific cargo in the same vesicle population. Recent advances in quantitative lipidomics [135] should also shed light on the nature of lipid segregation into different vesicle populations as well as a more basic understanding of bulk lipid egress from the ER.

Recent insight into another challenge for the COPII coat has arisen from identification of TANGO1 and cTAGE5, two accessory proteins that coordinate the COPII-mediated export of large collagen rods [62, 95]. These proteins present the first mechanism for accommodating large and bulky cargo into COPII vesicles although this does not seem to be a generalized mechanism. Large lipoproteins, which are similar in size to collagen, require only Sar1 teamed with several other proteins as the driving force for vesicle formation [118, 120, 121]. It is possible that the unique properties of the cargo such as structure, size and chemical composition could influence the requirements for forming export vesicles. For instance, a coat containing Sec23/Lst1 is known to produce larger vesicles than a Sec23/24-only coat, and this variation is attributed to the Lst1 selectivity for Pma1, a cargo that forms large oligomeric complexes [69]. Future studies on transport of large or usual molecules could focus on how coat composition could be tailored to the unique properties of the cargo in order to sculpt these 'mega vesicles' from the ER membrane.

Although we know a great deal of the minimal requirements for COPII-mediated ER export, much is yet to be learned. In particular, what are the processes that regulate individual COPII components and how do they work in concert? COPII vesicle biogenesis is initiated by the activation of Sar1 by its GEF, Sec12, but how is this triggered and at what point are the subsequent COPII players including Sec23/24 recruited? Evidence is increasing that posttranslational modification of individual coat components also regulates COPII-mediated ER export. Four of the core COPII components, Sec23, Sec24, Sec31 and Sec16, are known to be modified in some way [26, 136–140]. Experiments more than a decade ago revealed broad defects in COPII vesicle budding upon addition of the kinase inhibitors H89 [141] and the in vitro treatment of Sec31 with alkaline-phosphatase [137]. However, the molecular effects of these modifications remains unknown. Phosphorylation of Sec23 by Hrr25, a Golgi-associated kinase, has been speculated to regulate binding of COPII vesicles to the

Golgi in yeast, ensuring directionality of vesicle traffic by modulating sequential interactions of Sec23 with Sar1 and TRAPP [26]. Clearly, despite our detailed mechanistic understanding of this remarkable coat, a great deal remains to be understood about its regulation and its diversity in vivo.

Acknowledgments

Work in the Miller lab is supported by NIH grants GM085089 and GM078186.

Abbreviations

CLSD	cranio-lenticulo-sutural dysplasia
GEF	guanine nucleotide exchange factor
GAP	GTPase-activating protein
ERES	ER exit sites
tER	transitional ER
VSV-G	vesicular stomatitis virus glycoprotein
VTCs	vesicular tubular clusters
ERGIC	ER-Golgi intermediate compartment
SNARE	soluble N-ethylmaleimide attachment receptor
GPI-APs	glycophosphatidylinositol-anchored proteins
TANGO1	transport and golgi organization 1
cTAGE5	cutaneous T-cell lymphoma-associated antigen 5
PRD	proline rich domain
UPR	unfolded protein response
FA	fatty acid
VLDL	very low-density lipoprotein
FABP	fatty acid binding protein
apo	apolipoprotein
PCTV	prechylomicron transport vesicle
VAMP7	vesicle-associated membrane protein 7
L-FABP	liver FABP
VTVs	VLDL transport vesicles
MCS	membrane contact sites
LTPs	lipid transfer proteins
Star	steroidogenic acute regulatory

References

1. Bannykh SI, Balch WE. Membrane dynamics at the endoplasmic reticulum-Golgi interface. *J Cell Biol.* 1997; 138:1–4. [PubMed: 9214376]
2. Palade G. Intracellular aspects of the process of protein synthesis. *Science.* 1975; 189:867. [PubMed: 17812524]

3. Martínez-Menárguez JA, Geuze HJ, Slot JW, Klumperman J. Vesicular tubular clusters between the ER and Golgi mediate concentration of soluble secretory proteins by exclusion from COPI-coated vesicles. *Cell*. 1999; 98:81–90. [PubMed: 10412983]
4. Barlowe C, Orci L, Yeung T, Hosobuchi M, Hamamoto S, Salama N, Rexach MF, Ravazzola M, Amherdt M, Schekman R. COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. *Cell*. 1994; 77:895–907. [PubMed: 8004676]
5. Lee MCS, Miller EA, Goldberg J, Orci L, Schekman R. Bi-directional protein transport between the ER and Golgi. *Annu Rev Cell Dev Biol*. 2004; 20:87–123. [PubMed: 15473836]
6. Boyadjiev SA, Fromme JC, Ben J, Chong SS, Nauta C, Hur DJ, Zhang G, Hamamoto S, Schekman R, Ravazzola M, Orci L, Eyaid W. Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. *Nat Genet*. 2006; 38:1192–1197. [PubMed: 16980979]
7. Jones B, Jones EL, Bonney SA, Patel HN, Mensenkamp AR, Eichenbaum-Voline S, Rudling M, Myrdal U, Annesi G, Naik S, Meadows N, Quattrone A, Islam SA, Naoumova RP, Angelin B, Infante R, Levy E, Roy CC, Freemont PS, Scott J, Shoulders CC. Mutations in a Sar1 GTPase of COPII vesicles are associated with lipid absorption disorders. *Nat Genet*. 2003; 34:29–31. [PubMed: 12692552]
8. Schwarz K, Iolascon A, Verissimo F, Trede NS, Horsley W, Chen W, Paw BH, Hopfner KP, Holzmann K, Russo R, Esposito MR, Spano D, De Falco L, Heinrich K, Joggerst B, Rojewski MT, Perrotta S, Denecke J, Pannicke U, Delaunay J, Pepperkok R, Heimpel H. Mutations affecting the secretory COPII coat component SEC23B cause congenital dyserythropoietic anemia type II. *Nat Genet*. 2009; 41:936–940. [PubMed: 19561605]
9. Bianchi P, Fermo E, Vercellati C, Boschetti C, Barcellini W, Iurlo A, Marcello AP, Righetti PG, Zanella A. Congenital dyserythropoietic anemia type II (CDAIL) is caused by mutations in the SEC23B gene. *Hum Mutat*. 2009; 30:1292–1298. [PubMed: 19621418]
10. Barlowe C. Signals for COPII-dependent export from the ER: what's the ticket out? *Trends Cell Biol*. 2003; 13:295–300. [PubMed: 12791295]
11. Huang M, Weissman JT, Beraud-Dufour S, Luan P, Wang C, Chen W, Aridor M, Wilson IA, Balch WE. Crystal structure of Sar1-GDP at 1.7 Å resolution and the role of the NH2 terminus in ER export. *J Cell Biol*. 2001; 155:937–948. [PubMed: 11739406]
12. Bi X, Corpina RA, Goldberg J. Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat. *Nature*. 2002; 419:271–277. [PubMed: 12239560]
13. Lee MCS, Orci L, Hamamoto S, Futai E, Ravazzola M, Schekman R. Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. *Cell*. 2005; 122:605–617. [PubMed: 16122427]
14. Aridor M, Fish KN, Bannykh S, Weissman J, Roberts TH, Lippincott-Schwartz J, Balch WE. The Sar1 GTPase coordinates biosynthetic cargo selection with endoplasmic reticulum export site assembly. *J Cell Biol*. 2001; 152:213–229. [PubMed: 11149932]
15. Fromme JC, Ravazzola M, Hamamoto S, Al-Balwi M, Eyaid W, Boyadjiev SA, Cosson P, Schekman R, Orci L. The genetic basis of a craniofacial disease provides insight into COPII coat assembly. *Dev Cell*. 2007; 13:623–634. [PubMed: 17981132]
16. Bielli A, Haney CJ, Gabreski G, Watkins SC, Bannykh SI, Aridor M. Regulation of Sar1 NH2 terminus by GTP binding and hydrolysis promotes membrane deformation to control COPII vesicle fission. *The Journal of Cell Biology*. 2005; 171:919–924. [PubMed: 16344311]
17. Matsuoka K, Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R, Yeung T. COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. *Cell*. 1998; 93:263–275. [PubMed: 9568718]
18. Yoshihisa T, Barlowe C, Schekman R. Requirement for a GTPase-activating protein in vesicle budding from the endoplasmic reticulum. *Science*. 1993; 259:1466–1468. [PubMed: 8451644]
19. Bi X, Mancias JD, Goldberg J. Insights into COPII coat nucleation from the structure of Sec23.Sar1 complexed with the active fragment of Sec31. *Dev Cell*. 2007; 13:635–645. [PubMed: 17981133]
20. Russell, C.; Staggs, SM. *Traffic*. Copenhagen; Denmark: 2009. New Insights into the Structural Mechanisms of the COPII Coat.

21. Antonny B, Madden D, Hamamoto S, Orci L, Schekman R. Dynamics of the COPII coat with GTP and stable analogues. *Nat Cell Biol.* 2001; 3:531–537. [PubMed: 11389436]
22. Futai E, Hamamoto S, Orci L, Schekman R. GTP/GDP exchange by Sec12p enables COPII vesicle bud formation on synthetic liposomes. *EMBO J.* 2004; 23:4146–4155. [PubMed: 15457212]
23. Sato K, Nakano A. Dissection of COPII subunit-cargo assembly and disassembly kinetics during Sar1p-GTP hydrolysis. *Nat Struct Mol Biol.* 2005; 12:167–174. [PubMed: 15665868]
24. Forster R, Weiss M, Zimmermann T, Reynaud EG, Verissimo F, Stephens DJ, Pepperkok R. Secretory cargo regulates the turnover of COPII subunits at single ER exit sites. *Curr Biol.* 2006; 16:173–179. [PubMed: 16431369]
25. Cai H, Yu S, Menon S, Cai Y, Lazarova D, Fu C, Reinisch K, Hay JC, Ferro-Novick S. TRAPPI tethers COPII vesicles by binding the coat subunit Sec23. *Nature.* 2007; 445:941–944. [PubMed: 17287728]
26. Lord C, Bhandari D, Menon S, Ghassemian M, Nycz D, Hay J, Ghosh P, Ferro-Novick S. Sequential interactions with Sec23 control the direction of vesicle traffic. *Nature.* 2011
27. Tabata K, Sato K, Ide T, Nishizaka T, Nakano A, Noji H. Visualization of cargo concentration by COPII minimal machinery in a planar lipid membrane. *EMBO J.* 2009
28. Shimoi W, Ezawa I, Nakamoto K, Uesaki S, Gabreski G, Aridor M, Yamamoto A, Nagahama M, Tagaya M, Tani K. p125 is localized in endoplasmic reticulum exit sites and involved in their organization. *J Biol Chem.* 2005; 280:10141–10148. [PubMed: 15623529]
29. Shibata H, Suzuki H, Yoshida H, Maki M. ALG-2 directly binds Sec31A and localizes at endoplasmic reticulum exit sites in a Ca²⁺-dependent manner. *Biochem Biophys Res Commun.* 2007; 353:756–763. [PubMed: 17196169]
30. Supek F, Madden DT, Hamamoto S, Orci L, Schekman R. Sec16p potentiates the action of COPII proteins to bud transport vesicles. *The Journal of Cell Biology.* 2002; 158:1029–1038. [PubMed: 12235121]
31. Witte K, Schuh AL, Hegermann J, Sarkeshik A, Mayers JR, Schwarze K, Yates JR III, Eimer S, Audhya A. TFG-1 function in protein secretion and oncogenesis. *Nat Cell Biol.* 2011
32. Bannykh SI, Rowe T, Balch WE. The organization of endoplasmic reticulum export complexes. *The Journal of Cell Biology.* 1996; 135:19–35. [PubMed: 8858160]
33. Stephens DJ, Lin-Marq N, Pagano A, Pepperkok R, Paccard JP. COPI-coated ER-to-Golgi transport complexes segregate from COPII in close proximity to ER exit sites. *Journal of Cell Science.* 2000; 113(Pt 12):2177–2185. [PubMed: 10825291]
34. Watson P, Townley AK, Koka P, Palmer KJ, Stephens DJ. Sec16 defines endoplasmic reticulum exit sites and is required for secretory cargo export in mammalian cells. *Traffic (Copenhagen, Denmark).* 2006; 7:1678–1687.
35. Bhattacharyya D, Glick BS. Two mammalian Sec16 homologues have nonredundant functions in endoplasmic reticulum (ER) export and transitional ER organization. *Mol Biol Cell.* 2007; 18:839–849. [PubMed: 17192411]
36. Rossanese OW, Soderholm J, Bevis BJ, Sears IB, O'Connor J, Williamson EK, Glick BS. Golgi structure correlates with transitional endoplasmic reticulum organization in *Pichia pastoris* and *Saccharomyces cerevisiae*. *The Journal of Cell Biology.* 1999; 145:69–81. [PubMed: 10189369]
37. Blumental-Perry A, Haney CJ, Weixel KM, Watkins SC, Weisz OA, Aridor M. Phosphatidylinositol 4-phosphate formation at ER exit sites regulates ER export. *Dev Cell.* 2006; 11:671–682. [PubMed: 17084359]
38. Castillon GA, Watanabe R, Taylor M, Schwabe TME, Riezman H. Concentration of GPI-anchored proteins upon ER exit in yeast. *Traffic.* 2009; 10:186–200. [PubMed: 19054390]
39. Muñoz M, Morsomme P, Riezman H. Protein sorting upon exit from the endoplasmic reticulum. *Cell.* 2001; 104:313–320. [PubMed: 11207371]
40. Peng R, De Antoni A, Gallwitz D. Evidence for overlapping and distinct functions in protein transport of coat protein Sec24p family members. *J Biol Chem.* 2000; 275:11521–11528. [PubMed: 10753972]
41. Espenshade P, Gimeno RE, Holzmacher E, Teung P, Kaiser CA. Yeast SEC16 gene encodes a multidomain vesicle coat protein that interacts with Sec23p. *The Journal of Cell Biology.* 1995; 131:311–324. [PubMed: 7593161]

42. Shaywitz DA, Espenshade PJ, Gimeno RE, Kaiser CA. COPII subunit interactions in the assembly of the vesicle coat. *J Biol Chem.* 1997; 272:25413–25416. [PubMed: 9325247]
43. Miller EA, Barlowe C. Regulation of coat assembly-sorting things out at the ER. *Curr Opin Cell Biol.* 2010
44. Farhan H, Wendeler MW, Mitrovic S, Fava E, Silberberg Y, Sharan R, Zerial M, Hauri H-P. MAPK signaling to the early secretory pathway revealed by kinase/phosphatase functional screening. *The Journal of Cell Biology.* 2010; 189:997–1011. [PubMed: 20548102]
45. Zacharogianni M, Kondylis V, Tang Y, Farhan H, Xanthakis D, Fuchs F, Boutros M, Rabouille C. ERK7 is a negative regulator of protein secretion in response to amino-acid starvation by modulating Sec16 membrane association. *EMBO J.* 2011; 30:3684–3700. [PubMed: 21847093]
46. Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS. Global analysis of protein expression in yeast. *Nature.* 2003; 425:737–741. [PubMed: 14562106]
47. Dancourt J, Barlowe C. Protein sorting receptors in the early secretory pathway. *Annu Rev Biochem.* 2010; 79:777–802. [PubMed: 20533886]
48. Routledge KE, Gupta V, Balch WE. Emergent properties of proteostasis-COPII coupled systems in human health and disease. *Mol Membr Biol.* 2010; 27:385–397. [PubMed: 21054154]
49. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol.* 2007; 8:519–529. [PubMed: 17565364]
50. Miller EA, Beilharz TH, Malkus PN, Lee MCS, Hamamoto S, Orci L, Schekman R. Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell.* 2003; 114:497–509. [PubMed: 12941277]
51. Mossessova E, Bickford LC, Goldberg J. SNARE selectivity of the COPII coat. *Cell.* 2003; 114:483–495. [PubMed: 12941276]
52. Malkus P, Jiang F, Schekman R. Concentrative sorting of secretory cargo proteins into COPII-coated vesicles. *The Journal of Cell Biology.* 2002; 159:915–921. [PubMed: 12499351]
53. Kappeler F, Klopfenstein DR, Foguet M, Paccaud JP, Hauri HP. The recycling of ERGIC-53 in the early secretory pathway. ERGIC-53 carries a cytosolic endoplasmic reticulum-exit determinant interacting with COPII. *J Biol Chem.* 1997; 272:31801–31808. [PubMed: 9395526]
54. Nishimura N, Balch WE. A di-acidic signal required for selective export from the endoplasmic reticulum. *Science.* 1997; 277:556–558. [PubMed: 9228004]
55. Nufer O, Guldbrandsen S, Degen M, Kappeler F, Paccaud JP, Tani K, Hauri HP. Role of cytoplasmic C-terminal amino acids of membrane proteins in ER export. *J Cell Sci.* 2002; 115:619–628. [PubMed: 11861768]
56. Sucic S, El-Kasaby A, Kudlacek O, Sarker S, Sitte HH, Marin P, Freissmuth M. The serotonin transporter is an exclusive client of the COPII component SEC24C. *J Biol Chem.* 2011
57. Votsmeier C, Gallwitz D. An acidic sequence of a putative yeast Golgi membrane protein binds COPII and facilitates ER export. *EMBO J.* 2001; 20:6742–6750. [PubMed: 11726510]
58. Nufer O, Kappeler F, Guldbrandsen S, Hauri HP. ER export of ERGIC-53 is controlled by cooperation of targeting determinants in all three of its domains. *J Cell Sci.* 2003; 116:4429–4440. [PubMed: 13130098]
59. Schindler R, Itin C, Zerial M, Lottspeich F, Hauri HP. ERGIC-53, a membrane protein of the ER-Golgi intermediate compartment, carries an ER retention motif. *Eur J Cell Biol.* 1993; 61:1–9. [PubMed: 8223692]
60. Nichols WC, Ginsburg D. From the ER to the golgi: insights from the study of combined factors V and VIII deficiency. *Am J Hum Genet.* 1999; 64:1493–1498. [PubMed: 10330336]
61. Itin C, Schindler R, Hauri HP. Targeting of protein ERGIC-53 to the ER/ERGIC/cis-Golgi recycling pathway. *J Cell Biol.* 1995; 131:57–67. [PubMed: 7559786]
62. Saito K, Chen M, Bard F, Chen S, Zhou H, Woodley D, Polischuk R, Schekman R, Malhotra V. TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites. *Cell.* 2009; 136:891–902. [PubMed: 19269366]
63. Buchanan R, Kaufman A, Kung-Tran L, Miller EA. Genetic analysis of yeast Sec24p mutants suggests cargo binding is not co-operative during ER export. *Traffic (Copenhagen, Denmark).* 2010; 11:1034–1043.

64. Mancias JD, Goldberg J. Structural basis of cargo membrane protein discrimination by the human COPII coat machinery. *EMBO J.* 2008; 27:2918–2928. [PubMed: 18843296]
65. Merte J, Jensen D, Wright K, Sarsfield S, Wang Y, Schekman R, Ginty D. Sec24b selectively sorts Vangl2 to regulate planar cell polarity during neural tube closure. *Nat Cell Biol.* 2009
66. Wansleeben C, Feitsma H, Montcouquiol M, Kroon C, Cuppen E, Meijlink F. Planar cell polarity defects and defective Vangl2 trafficking in mutants for the COPII gene Sec24b. *Development.* 2010; 137:1067–1073. [PubMed: 20215345]
67. Kurihara T, Hamamoto S, Gimeno RE, Kaiser CA, Schekman R, Yoshihisa T. Sec24p and Iss1p function interchangeably in transport vesicle formation from the endoplasmic reticulum in *Saccharomyces cerevisiae*. *Mol Biol Cell.* 2000; 11:983–998. [PubMed: 10712514]
68. Miller E, Antonny B, Hamamoto S, Schekman R. Cargo selection into COPII vesicles is driven by the Sec24p subunit. *EMBO J.* 2002; 21:6105–6113. [PubMed: 12426382]
69. Shimoni Y, Kurihara T, Ravazzola M, Amherdt M, Orci L, Schekman R. Lst1p and Sec24p cooperate in sorting of the plasma membrane ATPase into COPII vesicles in *Saccharomyces cerevisiae*. *The Journal of Cell Biology.* 2000; 151:973–984. [PubMed: 11086000]
70. Lee MCS, Hamamoto S, Schekman R. Ceramide biosynthesis is required for the formation of the oligomeric H⁺-ATPase Pma1p in the yeast endoplasmic reticulum. *J Biol Chem.* 2002; 277:22395–22401. [PubMed: 11950838]
71. Fujita M, Kinoshita T. Structural remodeling of GPI anchors during biosynthesis and after attachment to proteins. *FEBS Lett.* 2010; 584:1670–1677. [PubMed: 19883648]
72. Conzelmann A, Riezman H, Desponds C, Bron C. A major 125-kd membrane glycoprotein of *Saccharomyces cerevisiae* is attached to the lipid bilayer through an inositol-containing phospholipid. *The EMBO Journal.* 1988; 7:2233–2240. [PubMed: 3046936]
73. Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell.* 1992; 68:533–544. [PubMed: 1531449]
74. Schroeder R, London E, Brown D. Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior. *Proc Natl Acad Sci USA.* 1994; 91:12130–12134. [PubMed: 7991596]
75. Mayor S, Sabharanjak S, Maxfield FR. Cholesterol-dependent retention of GPI-anchored proteins in endosomes. *EMBO J.* 1998; 17:4626–4638. [PubMed: 9707422]
76. Watanabe R, Funato K, Venkataraman K, Futerman AH, Riezman H. Sphingolipids are required for the stable membrane association of glycosylphosphatidylinositol-anchored proteins in yeast. *J Biol Chem.* 2002; 277:49538–49544. [PubMed: 12393888]
77. Horvath A, Sütterlin C, Manning-Krieg U, Movva NR, Riezman H. Ceramide synthesis enhances transport of GPI-anchored proteins to the Golgi apparatus in yeast. *The EMBO Journal.* 1994; 13:3687–3695. [PubMed: 8070398]
78. Schimmöller F, Singer-Krüger B, Schröder S, Krüger U, Barlowe C, Riezman H. The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi. *The EMBO Journal.* 1995; 14:1329–1339. [PubMed: 7729411]
79. Bonnon C, Wendeler MW, Paccard J-P, Hauri H-P. Selective export of human GPI-anchored proteins from the endoplasmic reticulum. *J Cell Sci.* 2010; 123:1705–1715. [PubMed: 20427317]
80. Takida S, Maeda Y, Kinoshita T. Mammalian GPI-anchored proteins require p24 proteins for their efficient transport from the ER to the plasma membrane. *Biochem J.* 2008; 409:555–562. [PubMed: 17927562]
81. Castillon GA, Aguilera-Romero A, Manzano-Lopez J, Epstein S, Kajiwar K, Funato K, Watanabe R, Riezman H, Muñoz M. The yeast p24 complex regulates GPI-anchored protein transport and quality control by monitoring anchor remodeling. *Mol Biol Cell.* 2011; 22:2924–2936. [PubMed: 21680708]
82. Muñoz M, Nuoffer C, Hauri HP, Riezman H. The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. *The Journal of Cell Biology.* 2000; 148:925–930. [PubMed: 10704443]

83. Schimmöller F, Singer-Krüger B, Schröder S, Krüger U, Barlowe C, Riezman H. The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi. *The EMBO Journal*. 1995; 14:1329–1339. [PubMed: 7729411]
84. Belden WJ, Barlowe C. Distinct roles for the cytoplasmic tail sequences of Emp24p and Erv25p in transport between the endoplasmic reticulum and Golgi complex. *J Biol Chem*. 2001; 276:43040–43048. [PubMed: 11560939]
85. Belden WJ, Barlowe C. Erv25p, a component of COPII-coated vesicles, forms a complex with Emp24p that is required for efficient endoplasmic reticulum to Golgi transport. *J Biol Chem*. 1996; 271:26939–26946. [PubMed: 8900179]
86. Marzioch M, Henthorn DC, Herrmann JM, Wilson R, Thomas DY, Bergeron JJ, Solari RC, Rowley A. Erp1p and Erp2p, partners for Emp24p and Erv25p in a yeast p24 complex. *Mol Biol Cell*. 1999; 10:1923–1938. [PubMed: 10359606]
87. Elrod-Erickson MJ, Kaiser CA. Genes that control the fidelity of endoplasmic reticulum to Golgi transport identified as suppressors of vesicle budding mutations. *Mol Biol Cell*. 1996; 7:1043–1058. [PubMed: 8862519]
88. Rodriguez-Boulán E, Powell SK. Polarity of epithelial and neuronal cells. *Annu Rev Cell Biol*. 1992; 8:395–427. [PubMed: 1476804]
89. Fujita M, Maeda Y, Ra M, Yamaguchi Y, Taguchi R, Kinoshita T. GPI glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. *Cell*. 2009; 139:352–365. [PubMed: 19837036]
90. Fujita M, Watanabe R, Jaensch N, Romanova-Michaelides M, Satoh T, Kato M, Riezman H, Yamaguchi Y, Maeda Y, Kinoshita T. Sorting of GPI-anchored proteins into ER exit sites by p24 proteins is dependent on remodeled GPI. *The Journal of Cell Biology*. 2011; 194:61–75. [PubMed: 21727194]
91. Stagg SM, LaPointe P, Razvi A, Gürkan C, Potter CS, Carragher B, Balch WE. Structural basis for cargo regulation of COPII coat assembly. *Cell*. 2008; 134:474–484. [PubMed: 18692470]
92. Antonny B, Gounon P, Schekman R, Orci L. Self-assembly of minimal COPII cages. *EMBO Rep*. 2003; 4:419–424. [PubMed: 12671686]
93. Stephens DJ, Pepperkok R. Imaging of procollagen transport reveals COPI-dependent cargo sorting during ER-to-Golgi transport in mammalian cells. *J Cell Sci*. 2002; 115:1149–1160. [PubMed: 11884515]
94. Boyadjiev SA, Justice CM, Eyaid W, McKusick VA, Lachman RS, Chowdry AB, Jabak M, Zwaan J, Wilson AF, Jabs EW. A novel dysmorphic syndrome with open calvarial sutures and sutural cataracts maps to chromosome 14q13-q21. *Hum Genet*. 2003; 113:1–9. [PubMed: 12677423]
95. Saito K, Yamashiro K, Ichikawa Y, Erlmann P, Kontani K, Malhotra V, Katada T. cTAGE5 mediates collagen secretion through interaction with TANGO1 at endoplasmic reticulum exit sites. *Mol Biol Cell*. 2011
96. Malhotra V, Erlmann P. Protein export at the ER: loading big collagens into COPII carriers. *EMBO J*. 2011; 30:3475–3480. [PubMed: 21878990]
97. Wieland FT, Gleason ML, Serafini TA, Rothman JE. The rate of bulk flow from the endoplasmic reticulum to the cell surface. *Cell*. 1987; 50:289–300. [PubMed: 3594573]
98. Thor F, Gautschi M, Geiger R, Helenius A. Bulk Flow Revisited: Transport of a Soluble Protein in the Secretory Pathway. *Traffic*. 2009
99. Lynch-Day MA, Bhandari D, Menon S, Huang J, Cai H, Bartholomew CR, Brumell JH, Ferro-Novick S, Klionsky DJ. Trs85 directs a Ypt1 GEF, TRAPP3, to the phagophore to promote autophagy. *Proc Natl Acad Sci USA*. 2010; 107:7811–7816. [PubMed: 20375281]
100. Bernales S, Schuck S, Walter P. ER-phagy: selective autophagy of the endoplasmic reticulum. *Autophagy*. 2007; 3:285–287. [PubMed: 17351330]
101. Nickel W, Rabouille C. Mechanisms of regulated unconventional protein secretion. *Nat Rev Mol Cell Biol*. 2009; 10:148–155. [PubMed: 19122676]
102. Karhinen L, Bastos RN, Jokitalo E, Makarow M. Endoplasmic reticulum exit of a secretory glycoprotein in the absence of sec24p family proteins in yeast. *Traffic*. 2005; 6:562–574. [PubMed: 15941408]

103. Fatal N, Suntio T, Makarow M. Selective protein exit from yeast endoplasmic reticulum in absence of functional COPII coat component Sec13p. *Mol Biol Cell*. 2002; 13:4130–4140. [PubMed: 12475940]
104. Fatal N, Karhinen L, Jokitalo E, Makarow M. Active and specific recruitment of a soluble cargo protein for endoplasmic reticulum exit in the absence of functional COPII component Sec24p. *J Cell Sci*. 2004; 117:1665–1673. [PubMed: 15075228]
105. Lev S. Non-vesicular lipid transport by lipid-transfer proteins and beyond. *Nat Rev Mol Cell Biol*. 2010; 11:739–750. [PubMed: 20823909]
106. Ikonen E. Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol*. 2008; 9:125–138. [PubMed: 18216769]
107. Radhakrishnan A, Goldstein JL, McDonald JG, Brown MS. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. *Cell Metab*. 2008; 8:512–521. [PubMed: 19041766]
108. Simons K, Gerl MJ. Revitalizing membrane rafts: new tools and insights. *Nat Rev Mol Cell Biol*. 2010; 11:688–699. [PubMed: 20861879]
109. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science*. 2010; 327:46–50. [PubMed: 20044567]
110. Gulati S, Liu Y, Munkacsı AB, Wilcox L, Sturley SL. Sterols and sphingolipids: Dynamic duo or partners in crime? *Prog Lipid Res*. 2010; 49:353–365. [PubMed: 20362613]
111. Young WW Jr, Lutz MS, Blackburn WA. Endogenous glycosphingolipids move to the cell surface at a rate consistent with bulk flow estimates. *J Biol Chem*. 1992; 267:12011–12015. [PubMed: 1601871]
112. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol*. 2008; 9:112–124. [PubMed: 18216768]
113. Bretscher MS, Munro S. Cholesterol and the Golgi apparatus. *Science*. 1993; 261:1280–1281. [PubMed: 8362242]
114. Baumann NA, Sullivan DP, Ohvo-Rekila H, Simonot C, Pottekat A, Klaassen Z, Beh CT, Menon AK. Transport of newly synthesized sterol to the sterol-enriched plasma membrane occurs via nonvesicular equilibration. *Biochemistry*. 2005; 44:5816–5826. [PubMed: 15823040]
115. Mansbach CM, Siddiqi SA. The biogenesis of chylomicrons. *Annu Rev Physiol*. 2010; 72:315–333. [PubMed: 20148678]
116. Lo C-M, Nordskog BK, Nauli AM, Zheng S, Vonlehmden SB, Yang Q, Lee D, Swift LL, Davidson NO, Tso P. Why does the gut choose apolipoprotein B48 but not B100 for chylomicron formation? *Am J Physiol Gastrointest Liver Physiol*. 2008; 294:G344–352. [PubMed: 18006607]
117. Gusarova V, Brodsky JL, Fisher EA. Apolipoprotein B100 exit from the endoplasmic reticulum (ER) is COPII-dependent, and its lipidation to very low density lipoprotein occurs post-ER. *J Biol Chem*. 2003; 278:48051–48058. [PubMed: 12960170]
118. Siddiqi SA. VLDL exits from the endoplasmic reticulum in a specialized vesicle, the VLDL transport vesicle, in rat primary hepatocytes. *Biochem J*. 2008; 413:333–342. [PubMed: 18397176]
119. Siddiqi S, Siddiqi SA, Mansbach CM. Sec24C is required for docking the prechylomicron transport vesicle with the Golgi. *J Lipid Res*. 2010; 51:1093–1100. [PubMed: 19965600]
120. Siddiqi S, Saleem U, Abumrad NA, Davidson NO, Storch J, Siddiqi SA, Mansbach CM. A novel multiprotein complex is required to generate the prechylomicron transport vesicle from intestinal ER. *J Lipid Res*. 2010; 51:1918–1928. [PubMed: 20237389]
121. Neeli I, Siddiqi SA, Siddiqi S, Mahan J, Lagakos WS, Binas B, Gheyi T, Storch J, Mansbach CM. Liver fatty acid-binding protein initiates budding of pre-chylomicron transport vesicles from intestinal endoplasmic reticulum. *J Biol Chem*. 2007; 282:17974–17984. [PubMed: 17449472]
122. Siddiqi S, Mani AM, Siddiqi SA. The identification of the SNARE complex required for the fusion of VLDL-transport vesicle with hepatic cis-Golgi. *Biochem J*. 2010; 429:391–401. [PubMed: 20450495]
123. Kaplan MR, Simoni RD. Intracellular transport of phosphatidylcholine to the plasma membrane. *J Cell Biol*. 1985; 101:441–445. [PubMed: 4040519]

124. Vance JE, Aasman EJ, Szarka R. Brefeldin A does not inhibit the movement of phosphatidylethanolamine from its sites for synthesis to the cell surface. *J Biol Chem.* 1991; 266:8241–8247. [PubMed: 2022641]
125. Milla P, Athenstaedt K, Viola F, Oliaro-Bosso S, Kohlwein SD, Daum G, Balliano G. Yeast oxidosqualene cyclase (Erg7p) is a major component of lipid particles. *J Biol Chem.* 2002; 277:2406–2412. [PubMed: 11706015]
126. Voelker DR. Reconstitution of phosphatidylserine import into rat liver mitochondria. *J Biol Chem.* 1989; 264:8019–8025. [PubMed: 2542259]
127. Simbeni R, Paltauf F, Daum G. Intramitochondrial transfer of phospholipids in the yeast, *Saccharomyces cerevisiae*. *J Biol Chem.* 1990; 265:281–285. [PubMed: 2104619]
128. Vance JE. Phospholipid synthesis in a membrane fraction associated with mitochondria. *J Biol Chem.* 1990; 265:7248–7256. [PubMed: 2332429]
129. Levine T. Short-range intracellular trafficking of small molecules across endoplasmic reticulum junctions. *Trends Cell Biol.* 2004; 14:483–490. [PubMed: 15350976]
130. Hanada K, Kumagai K, Tomishige N, Yamaji T. CERT-mediated trafficking of ceramide. *Biochim Biophys Acta.* 2009; 1791:684–691. [PubMed: 19416656]
131. Alpy F, Tomasetto C. Give lipids a START: the StAR-related lipid transfer (START) domain in mammals. *Journal of Cell Science.* 2005; 118:2791–2801. [PubMed: 15976441]
132. Wüstner D, Herrmann A, Hao M, Maxfield FR. Rapid nonvesicular transport of sterol between the plasma membrane domains of polarized hepatic cells. *J Biol Chem.* 2002; 277:30325–30336. [PubMed: 12050151]
133. Li Y, Prinz WA. ATP-binding cassette (ABC) transporters mediate nonvesicular, raft-modulated sterol movement from the plasma membrane to the endoplasmic reticulum. *J Biol Chem.* 2004; 279:45226–45234. [PubMed: 15316012]
134. Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S, Brügger B, Ringler P, Müller SA, Rammner B, Gräter F, Hub JS, De Groot BL, Mieskes G, Moriyama Y, Klingauf J, Grubmüller H, Heuser J, Wieland F, Jahn R. Molecular anatomy of a trafficking organelle. *Cell.* 2006; 127:831–846. [PubMed: 17110340]
135. Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc Natl Acad Sci U S A.* 2009; 106:2136–2141. [PubMed: 19174513]
136. Sharpe LJ, Luu W, Brown AJ. Akt phosphorylates Sec24: New clues into the regulation of ER-to-Golgi trafficking. *Traffic (Copenhagen, Denmark).* 2010
137. Salama NR, Chuang JS, Schekman RW. Sec31 encodes an essential component of the COPII coat required for transport vesicle budding from the endoplasmic reticulum. *Mol Biol Cell.* 1997; 8:205–217. [PubMed: 9190202]
138. Dudognon P, Maeder-Garavaglia C, Carpentier J-L, Paccaud J-P. Regulation of a COPII component by cytosolic O-glycosylation during mitosis. *FEBS Lett.* 2004; 561:44–50. [PubMed: 15013749]
139. Cohen M, Stutz F, Belgareh N, Haguénauer-Tsapis R, Dargemont C. Ubp3 requires a cofactor, Bre5, to specifically de-ubiquitinate the COPII protein, Sec23. *Nat Cell Biol.* 2003; 5:661–667. [PubMed: 12778054]
140. Ossareh-Nazari B, Cohen M, Dargemont C. The Rsp5 ubiquitin ligase and the AAA-ATPase Cdc48 control the ubiquitin-mediated degradation of the COPII component Sec23. *Exp Cell Res.* 2010; 316:3351–3357. [PubMed: 20846524]
141. Aridor M, Balch WE. Kinase signaling initiates coat complex II (COPII) recruitment and export from the mammalian endoplasmic reticulum. *J Biol Chem.* 2000; 275:35673–35676. [PubMed: 11001944]

Highlights

Transport of lipids and proteins from the ER is a highly regulated process.

Protein exit from the ER occurs via signal-mediate export and bulk flow.

The COPII machinery can adapt to export large or bulky cargoes.

ER export of lipids occurs via both vesicular and non-vesicular mechanisms.

Figure 1. Cargo size influences the requirements for vesicle formation in the ER

(A) Secreted proteins exit the ER via small vesicles of 60–100 nm generated by the COPII coat complex – Sar1, Sec23/24, Sec13/31. The GEF, Sec12, exchanges GDP for GTP on Sar1, which triggers a conformational change and induces initial membrane curvature by insertion of an amphipathic helix into the ER membrane. When the COPII inner coat (Sec23/24) binds to Sar1, the Sec24 subunit recruits and concentrates protein cargo at ERES. The outer coat (Sec13/31) binds to the inner coat and polymerizes to form a cage-like structure providing sufficient force to deform the membrane into vesicles. Sec16, a ~250 kDa cytosolic accessory protein closely associated with the ER membrane, organizes ERES and interacts with all members of the COPII complex. (B) Large lipoprotein particles such as chylomicrons and VLDLs (~80–250 nm in diameter) are secreted from the cell into the bloodstream via specialized vesicles. In addition to COPII proteins, lipoprotein particles require several other proteins – liver-fatty acid binding protein (L-FABP), CD36 and VAMP7 for ER-Golgi transport, although the molecular details of the coat and its assembly have not been determined. (C) Collagen VII, a large protein cargo forming ~300–400 nm rods requires accessory proteins TANGO1 and cTAGE5 for efficient ER export via COPII vesicles. The TANGO1–cTAGE5 protein complex may stall recruitment of the outer COPII coat to permit generation of vesicles large enough to accommodate the long collagen rods. The TANGO1 SH3 domain binds to collagen in the lumen, while the proline-rich domains (PRDs) of both TANGO1 and cTAGE5 bind to the inner coat preventing Sec13/31 from binding, enabling the size of the vesicle to grow.

Figure 2. Cargo is selected by the COPII subunit, Sec24, and its homologs at ER exit sites
(A) The Sec24 subunit of the pre-budding complex (Sar1-Sec23/24) recruits a diverse range of cargo to COPII vesicles including transmembrane proteins (TM), soluble proteins via a cargo receptor and SNARE proteins, which are required for vesicle fusion with the Golgi. Signal sequences or epitopes on the cargo proteins and receptors interact with specific binding sites - the A, B or C site - on Sec24 (inset). **(B)** Some specific cargoes can be packaged into COPII vesicles by the Sec24 isoform, Lst1, including the ~100 kDa H⁺-ATPase, Pma1, that forms large oligomers, along with GPI-APs, Gas1, and GPI-AP cargo receptors, the p24 complex. GPI-APs are localized to membrane microdomains that are enriched in sphingolipids and sterols (inset, highlighted in pink), likely stabilizing the GPI anchor. The inability of Lst1 to package SNAREs, suggests COPII vesicles are generated as a combination of Sec24 and its paralogs.