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Protein Trafficking in the Biosynthetic Pathway

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Abstract

In the absence of specific mechanisms to recognize and sequester cargo destined for transport and retrieval, communicating organelles would quickly lose their identity. The biosynthetic pathway, comprising of the endoplasmic reticulum, the Golgi complex, vesicular-tubular clusters (also known as ERGIC or pre-Golgi intermediates) and vesicular/tubular transport carriers has been a frequent target for the examination of such transport processes. In the biosynthetic pathway, proteins are directed to the endoplasmic reticulum by an N-terminal signal peptide. Within the lumen of the ER, proteins may be proteolytically processed, folded, glycosylated and assembled into their tertiary or quaternary structures. Improperly folded proteins are passed by retrograde transport through the ER translocon to be degraded by a proteasome. Correctly folded proteins are packaged into vesicles or tubules at ER exit sites and transported to the Golgi apparatus where further maturation occurs. Though many questions remain regarding mechanisms of protein retention, sorting, packaging, and recycling in the biosynthetic pathway, new discoveries through complementary methods have assisted in the clarification of a number of the steps involved.

The Endoplasmic Reticulum

Structure and Function

The endoplasmic reticulum (ER), originally discovered by Porter and coworkers in 1945 (Palade 1975), is the first organelle in the biosynthetic pathway (Figure 1). The ER is a labyrinthine array of membrane-bounded tubules and cisternae which extend throughout the cell. The position and organization of the ER has been demonstrated to depend, at least in part, on microtubules (Lee *et al.,* 1989, Lane and Allan 1998, Marsh *et al.,* 2001). The functions of the ER include: protein synthesis, folding, assembly, and degradation; lipid biosynthesis and metabolism; detoxification; nucleus compartmentalization; ion gradient retention; and membrane transport (Rooney and Meldolesi 1996, Lippincott-Schwartz *et al.,* 2000). ER membranes form a continuous interconnected system of rough (RER) and smooth (SER) regions, depending on whether ribosomes are associated with their cytoplasmic surfaces (Cole *et al.,* 1996a, Dayel *et al.,* 1999). RER is the site of cotranslational or posttranslational protein insertion, while SER is proposed to be the site of lipid biosynthesis and detoxification (Amar-Costesec *et al.,* 1984, Lippincott-Schwartz *et al.,* 2000).

The RER represents the entry point for newly synthesized proteins destined for the biosynthetic and secretory pathways. The process of how the appropriate proteins are targeted to the ER, and thus, the true starting point of research on protein trafficking, is

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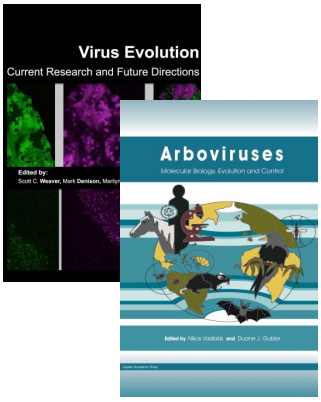

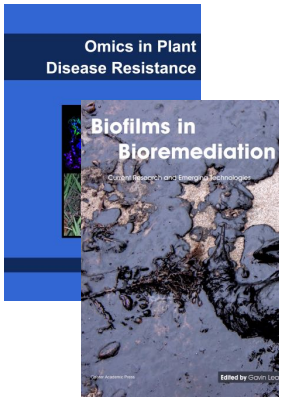

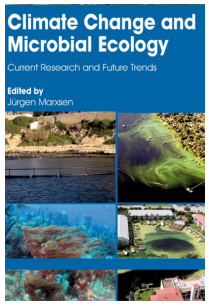

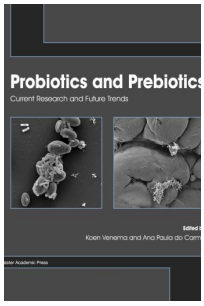

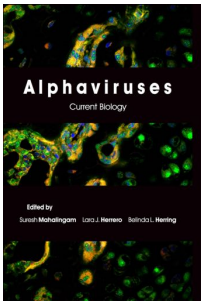

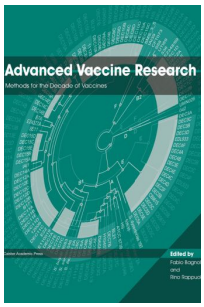

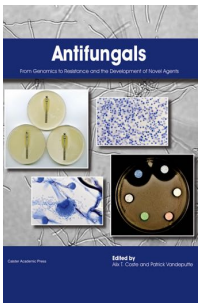

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explained by the signal hypothesis. This hypothesis postulates that an amino-terminal leader peptide serves as a signal that directs the protein to the ER and then is cleaved off by a signal peptidase in the ER membrane before the polypeptide chain is fully synthesized (Blobel and Dobberstein 1975, Walter and Johnson 1994). In mammalian cells, greater detail has been elucidated. The ER signal peptide on the nascent polypeptide chain is bound by the signal-recognition particle (SRP), which cycles between the ER membrane and the cytosol. The binding of the SRP to the signal peptide and ribosome induces a pause in translation and permits the binding of the SRP-ribosome complex to the SRP receptor, which is located adjacent to the translocon. The translocon is a complex molecular machine that regulates the movement of polypeptides in both directions through the ER bilayer while maintaining the membrane permeability barrier. Once the SRP-ribosome complex is bound to the SRP receptor, the nascent chain is inserted into the aqueous translocon pore and a GTP-dependent interaction of SRP with its receptor triggers the release of SRP from the ribosome and the SRP receptor from the translocon (Bacher *et al.,* 1996). The nascent chain is prevented by Binding Protein (BiP) from passing through the luminal end of the pore until the chain reaches ~ 70 amino acids in length (Crowley *et al.,* 1994). The delayed release of BiP and the final opening of the pore may constitute a safety mechanism to ensure that one end of the pore is not opened before the other end is completely sealed. The release of the BiP may be elicited after the unfolded nascent chain becomes long enough to bind to BiP, alter its conformation, and release BiP from the translocon. Cotranslational protein translocation then proceeds through the aqueous pore, which is now sealed at its cytoplasmic end by tight binding of the ribosome to the translocon. When the chain length totals ~150 residues, the signal peptidase cleaves off the leader peptide. After termination of translation, the ribosome is released and the pore contracts, sealed on its luminal side by BiP (Hamman *et al.,* 1998, Johnson and van Waes 1999). For soluble proteins, cleavage of the signal peptide releases the polypeptide into the ER lumen. Other proteins remain anchored to the phospholipid bilayer by a covalently attatched glycosylphosphatidylinositol (GPI) membrane anchor. These proteins are initially anchored to the ER membrane by an internal stop-transfer membrane anchor sequence. However, a short sequence of amino acids in the exoplasmic domain adjacent to the membrane-spanning domain is recognized by an endoprotease that simultaneously cleaves off the stop-transfer membrane-anchor sequence and transfers the remainder of the protein to a pre-formed GPI anchor in the membrane (Kodukula *et al.,* 1992). In contrast, single or multiple-pass integral membrane proteins remain in the ER membrane after cleavage by the signal peptidase due to the presence of uncleaved stop-transfer membrane-anchor sequences or internal signal anchor sequences. Because membrane proteins are always inserted from the cytosolic side of the ER in a programmed manner, all copies of the same polypeptide chain will have the same orientation in the lipid bilayer. This generates an asymmetrical ER membrane in which the protein domains exposed on one side differ from those exposed on the other. This asymmetry is maintained as proteins made in the ER are transported to other cell membranes (Lodish *et al.,* 2000).

Quality Control

Once in the ER, proteins are covalently modified (*N*-glycosylation, oligosaccharide trimming, formation of disulfide bonds) and acquire their proper tertiary and, in some cases, quaternary structures. Proper folding of proteins is facilitated in the ER by a number of chaperones (such as BiP, calnexin, calreticulin, etc.) and by enzymes able to catalyze disulfide bond formation (such as protein disulfide isomerase, ERp57, etc; Ellgaard *et al.,* 1999, Farmery *et al.,* 2000, High *et al.,* 2000, Molinari and Helenius 2000). The correct functioning of many of these folding-assisting proteins is dependent upon maintenance of the ER luminal calcium (Ca^{2+}) concentration and oxidizing environment (Meldolesi and Pozzan 1998). Proteins that

consistently fail to fold properly are initially retained in the ER, then eventually transported in a retrograde manner through the ER translocon to the cytosol where they are proteolytically degraded by a proteasome (Brodsky and McCracken 1997, Plemper *et al.,* 1997, Plemper and Wolf 1999). Proteasomes are highly conserved barrel-shaped proteolytic complexes that mediate the elimination of abnormal proteins as well as critical regulatory proteins that must be rapidly destroyed for normal growth and metabolism. Proteasomes generally degrade proteins to small peptides, most of which are hydrolyzed to amino acids by cytosolic exopeptidases. Some of the oligopeptides generated by proteasomes, however, are presented to the immune system on major histocompatibility complex (MHC) class I molecules (Rock *et al.,* 1994, Goldberg 1995, Niedermann *et al.,* 1997). The majority of proteins destined for degradation by the proteasome are marked by conjugation to multiple molecules of ubiquitin (Hershko and Ciechanover 1992). The first clear indication that a proteasome degrades ER proteins came from the examination of the cystic fibrosis transmembrane conductance regulator (CFTR), an integral membrane protein that facilitates chloride transport across the apical plasma membrane of epithelial cells (Brodsky and McCracken 1997). The most common mutation in individuals with cystic fibrosis is a deletion of phenylalanine at position 508 (F508), resulting in the retention of this protein in the ER because an ATP-dependent conformational change required for ER-to-Golgi transport is prevented (Lukacs *et al.,* 1994). It was demonstrated that the addition of lactacystin, a proteasome inhibitor, obstructed the degradation of ER-retained forms of CFTR and increased the population of polyubiquitinated CFTR derivatives (Jensen *et al.,* 1995). Recently, Meacham *et al.,* (2001) showed that CHIP (carboxyl terminus of Hsp70 interactin protein) functions with Hsc70 to sense the folded state of CFTR and targets aberrant forms for proteasomal degradation by promoting their ubiquitination. Therefore, when proteins exit the ER, they are not only sorted away from resident ER proteins, but also from conformational variants of themselves.

ER-to-Golgi Transport

Once properly folded and assembled, proteins are translocated from the ER to the Golgi apparatus in transport carriers that move along microtubules, likely driven by the minus end-directed motor complex of dynein/dynactin (Scales *et al.,* 1997, Lane and Allan 1998). Two models have been proposed to explain the nature of the export event from the ER. The first suggests that budding from the ER is a selective process, while the second views the event as a default process. Current data corroborates the first model. It has been noted that several cargo proteins are concentrated many-fold during the budding process (Mizuno and Singer 1993, Balch *et al.,* 1994), and that different proteins are exported from the ER at markedly different rates (Lodish 1988), thus supporting the existence of selectivity during ER export. Moreover, several integral membrane proteins implicated in the sorting of specific cargo molecules have been identified. For example, Shr3p, an ER resident membrane protein, is required for the incorporation of amino acid permeases Gap1p and Hip1p into COPII-(coatomer-like protein coat, see text below) coated vesicles, thus demonstrating selectivity in export from the ER (Kuehn *et al.,* 1996). In another example, Nichols *et al.,* (1998) showed that mutations in ERGIC-53, an integral membrane protein with lectin-like activity that cycles between the ER and Golgi, causes a combined deficiency in coagulation factors V and VIII, resulting in an autosomal recessive bleeding disorder, and thus, suggesting a role for ERGIC-53 in the selective export of these coagulation factors.

Proteins to be exported from the ER are partitioned at ER exit sites into coated transport carriers. These ER exit sites, also termed the transitional ER, are scattered over the surface of the ER and exist as membrane domains with an increased level of organization of protein and lipid adjacent to elaborate tubular clusters (also called pre-Golgi intermediates, vesicular-

tubular clusters, or ERGIC; Saraste and Kuismanen 1992, Bannykh *et al.,* 1996, Hauri *et al.,* 2000). ER exit sites observed at the electron microscope level usually contain multiple budding profiles, possibly representing budding vesicles that subsequently fuse locally to form vesicular-tubular clusters. However, because the membranes at ER exit sites sometimes show direct continuity with the vesicular-tubular clusters, an alternative possibility is that the budding profiles represent ER exit sites transforming directly into vesicular-tubular clusters without the need for small vesicle intermediates (Krijnse-Locker *et al.,* 1994, Stinchcombe *et al.,* 1995, Lippincott-Schwartz *et al.,* 2000). Presley *et al.,* (1997) fused GFP to the temperature-sensitive ts045 mutant of the vesicular stomatitis viral glycoprotein (VSVG) to study ER-to-Golgi transport in living cells. Upon export from the ER, VSVG-GFP became concentrated in many differently shaped, rapidly forming pre-Golgi structures. No loss of fluorescent material from these pre-Golgi structures occurred during their translocation to the Golgi complex, and they frequently stretched into tubular shapes, contrasting with the traditional view of small vesicles as the primary vehicles for ER-to-Golgi transport (see Quicktime movie at http: //dir.nichd.nih.gov/CBMB/pb1labob.html).

Cargo to be transported has been demonstrated to rely on the assembly of the cytosolic coat complex COPII onto ER exit sites. The COPII coat complex was first identified in yeast as distinct from both clathrin and coatomer coats (Barlowe *et al.,* 1994). Previously, a non-clathrin coat termed COP (coatomer protein) consisting of the cytosolic seven subunit protein coatomer as well as the GTPase ADP-ribosylation factor 1 (ARF1) that regulates both the sorting of cargo into COP vesicles and the assembly/disassembly of the coat was discovered to operate in the biosynthetic pathway (Malhotra *et al.,* 1989, Nickel and Wieland 1998). Like COP, COPII was also discovered as a non-clathrin coat that functioned in the biosynthetic pathway and relied on the activity of a small monomeric GTPase for coat assembly. Thus, COP was renamed "COPI" and the newly discovered coat was named "COPII" (Barlowe *et al.,* 1994). COPII was found to consist of five subunits: the Ras-related GTPase Sar1p and two heterodimeric complexes (Sec23p/24p and Sec13p/Sec31p; Schekman and Orci 1996). Later, Sec16p, a membrane-associated peripheral protein that may be involved in cargo selection and other regulatory events, was also identified as a coat component (Gimeno *et al.,* 1996, Hong 1998). Additionally, an integral ER membrane glycoprotein, Sec12p, was deemed necessary to guide the budding machinery to the proper compartment. Mammalian equivalents of the COPII subunits have been detected by molecular cloning and immunological cross-reaction. A COPII coat is assembled when Sec12p recruits Sar1p and catalyzes the exchange of GTP in place of GDP. Sec23p/Sec24p and Sec13p/Sec31p heterodimers are then, respectively, assembled onto the cytoplasmic leaflet of the activated ER membrane to form a COPII-coated bud (Figure 2; Schekman and Orci 1996). This bud was generally thought to transform into a vesicle, though it may not immediately sever, allowing a tubule formation that could cluster with adjacent COPII-derived tubules (Lippincott-Schwartz *et al.,* 2000). How specific proteins are recruited into COPII coats is an active topic of research. In the case of a number of integral membrane proteins (such as CFTR, glucose transporter 4, low-density lipoprotein receptor, epidermal growth factor receptor and nerve growth factor receptor), an Asp-X-Glu (DXE) motif may serve as the signal required for efficient recruitment into COPII vesicles leading to their selective export from the ER (Nishimura and Balch 1997). In the case of soluble proteins (such as invertase), a family of 24 kDa membrane proteins referred to as the p24 family have been shown to selectively bind soluble ER proteins destined for transport to the Golgi while excluding resident ER proteins such as Hsc20 (Lodish *et al.,* 2000, Muniz *et al.,* 2000).

COPII does not persist on the transport carriers throughout their passage to the Golgi apparatus. A sequential mode of action of COPII binding, followed by COPI binding, COPII

dissociation, and COPI persistence has been demonstrated (Aridor *et al.,* 1995, Rowe *et al.,* 1996, Scales *et al.,* 1997, Stephens *et al.,* 2000). This coat exchange is an early event that precedes the targeting of ER-derived vesicles to vesicular-tubular clusters(VTCs)/ERGIC (Rowe *et al.,* 1996, Stephens *et al.,* 2000). The subsequent binding of COPI implies a role in retrieving earlier-acting factors from the transport carriers (e.g. chaperones, COPII-associated proteins) which, if not removed, would prevent transfer of cargo to the Golgi apparatus (Scales *et al.,* 1997).

Retention and Retrieval of Resident Proteins

Each organelle in the biosynthetic and secretory pathways is required to selectively allow transit of newly synthesized proteins destined for export, while still maintaining a unique set of resident proteins that defines its structural and functional properties. In the case of the ER, residency is achieved in two ways: prevention of residents from entering transport vesicles, and retrieval of those residents that escape. Though retention of residents seems the most obvious method, how such proteins are retained in the ER remains enigmatic. Several mechanisms have been suggested. Retention of ER membrane proteins may be achieved by interaction of their cytoplasmic domains with cytoskeletal elements, such as microtubules (Dahllof *et al.,* 1991). Retention may also occur through the oligomerization of proteins into complexes that are kinetically or physically excluded from the budding vesicle (Hortsch and Meyer 1985, Teasdale and Jackson 1996). It has also been proposed that Ca^{2+} in the ER lumen serves as a stabilizer of a reticular matrix of resident soluble and membrane proteins, constituting an immobile phase through which secretory proteins percolate (Hammond and Helenius 1995). Evidence supporting this model comes from studies indicating that retrieval appears to be Ca^{2+}-independent (Wilson *et al.,* 1993), whereas perturbation of intracellular Ca^{2+} stores by ionophores induces secretion of resident proteins (Booth and Koch 1989). Recently, Andersson *et al.,* (1999) demonstrated that protein targeting to the ER by dilysine signals involves direct retention in addition to retrieval. Chimerae consisting of the plasma membrane protein CD4 and either a C-terminal Lys-Lys-Ala-Ala (KKAA) or a Lys-Lys-Phe-Phe (KKFF) signal were expressed in Lec-1 cells. The KKAA chimerae were localized to the ER by confocal microscopy, and were neither processed by *cis*-Golgi-specific enzymes *in vivo*, nor included into ER-derived transport vesicles in an *in vitro* binding assay, suggesting that the KKAA-bearing proteins were permanently retained in the ER.

Unlike retention, retrieval of resident proteins back to the ER has been well documented. A number of sorting signals that direct localization of resident proteins to the ER has been identified. The criteria defining organellar targeting motifs are that they function in a specific position of a protein, can be disrupted through mutation, and confer residency in a specific organelle when engineered onto a reporter protein. Retrieval of many soluble ER proteins has been demonstrated to rely on a C-terminal Lys-Asp-Glu-Leu signal (KDEL; HDEL in yeast). This signal is recognized by the Erd2p (endoplasmic reticulum retention defective complementation group 2) transmembrane protein receptor that resides at steady state in the Golgi apparatus (Munro and Pelham 1987, Lewis and Pelham 1990, Aoe *et al.,* 1997). Additionally, carboxy-terminal dilysine (KKXX, KXKXX) and amino-terminal diarginine (XXRR) sequences have been shown to function as retrieval signals for ER resident membrane proteins (Schutze *et al.,* 1994, Townsley and Pelham 1994, Teasdale and Jackson 1996). Both the luminal and cytoplasmic motifs are conserved among species and mediate rapid recycling from post-ER compartments as distal as the *trans* Golgi network (Miesenbock and Rothman 1995). The recycling of the ER residents bearing the aforementioned signals depends on COPI (Storrie *et al.,* 2000). As first demonstrated by Cosson and Letourneur (1994), the K(X)KXX motif binds directly to coatomer *in vitro*, and corresponding mutations

that abolish binding result in the loss of reporter molecules to the cell surface *in vivo*. Moreover, inhibition of COPI function through expression of mutated ARF1 or microinjection of antibodies to coatomer blocks recycling of both the KDEL receptor and ERGIC-53 from the Golgi to the ER (Girod *et al.,* 1999). These experiments clearly show the importance of COPI in retrograde traffic from the Golgi to the ER.

The Golgi Apparatus

Structure and Function

The Golgi apparatus, originally discovered by Camillo Golgi in 1898, consists of a collection of membrane-bounded cisternae surrounded by tubules and vesicles. The precise number of Golgi compartments is still debated, but most investigators recognize the following four: *cis* (also called *cis*-Golgi network or CGN), *medial, trans*, and *trans*-Golgi network (TGN; Griffiths and Simons 1986, Rambourg and Clermont 1990, Ladinsky *et al.,* 1994, Farquhar and Palade 1998). In most metazoan cells, a single Golgi apparatus is located adjacent to the nucleus, while in plants and fungi many Golgi apparatus are distributed throughout the cell (Griffing 1991, Lane and Allan 1998). In 1984, Saraste and Kuismanen reported the existence of a pre-Golgi compartment located between the transitional ER and *cis* Golgi. Recognition of distinct features of this possible compartment, now referred to as VTCs or ERGIC, came about after the discovery of the only known marker of this area, p53 (ERGIC53; Schweizer *et al.,* 1988) and its rat homologue p58 (ERGIC58; Saraste *et al.,* 1987, Lahtinen *et al.,* 1996). Currently, the question of whether VTCs/ERGIC is a true compartment or a transient membrane structure remains unanswered (Hauri and Schweizer 1992, Presley *et al.,* 1997, Scales *et al.,* 1997, Hauri *et al.,* 2000).

In animal cells, evidence implies that microtubules and the minus end-directed motor dynein are involved in Golgi positioning, since microtubule-depolymerizing drugs (colchicine, nocodazole) or overexpression of the dynamitin subunit of the dynactin complex results in Golgi fragmentation (Cole *et al.,* 1996b, Burkhardt *et al.,* 1997, Burkhardt 1998). Recently, it was reported that Hook proteins constitute a novel family of coiled-coil proteins that bind organelles to microtubules. More specifically, human Hook3 was implicated in binding the Golgi apparatus to microtubules, thus defining its architecture and localization (Walenta *et al.,* 2001).

In general, transport carriers with cargo derived from the ER deliver their contents uniquely to the *cis* face of the Golgi, which exists as a tubular network. The cargo then moves through the polarized stacks of flattened cisternae enriched in glycoprotein and glycolipid-processing enzymes to the *trans* Golgi network (TGN) where they are packaged into post-Golgi carriers before being delivered to their final destination (Palade 1975, Teasdale and Jackson 1996). The functions of the Golgi apparatus include: *N*-linked oligosaccharide processing, *O*-linked glycosylation, proteoglycan formation, sugar and tyrosine sulfation, lipid modification, and cargo sorting/filtering (Neutra and Leblond 1966, Tooze *et al.,* 1988, Munro 1998, Lodish *et al.,* 2000). Though some *N*-linked oligosaccharide processing does occur in the ER, the extreme structural diversity of *N*-linked oligosaccharides in mature glycoproteins of different organisms or of different cell types belonging to multicellular organisms originates by differential processing reactions in the Golgi apparatus (Parodi 2000).

Retention and Retrieval of Resident Proteins

The population of resident proteins and sorting components must be maintained despite the continuous flow of cargo through the Golgi apparatus. Furthermore, mechanisms must exist

to account for the restriction of resident enzymes and trafficking components such as SNAREs to specific cisternae. The first insights into the nature of these mechanisms were provided by Machamer and Rose (1987), who revealed the unexpected finding that targeting and retention of Golgi membrane proteins resides in a single transmembrane domain. Other studies since then have confirmed the importance of this domain as a localization signal (Colley 1997), but how this signal mediates retention is still unknown. Two models have been proposed to explain this retention mechanism: the kin-recognition model (Weisz *et al.,* 1993, Nilsson *et al.,* 1994, Slusarewicz *et al.,* 1994) and the bilayer-mediated sorting model (Bretscher and Munro 1993). The first model envisages that enzymes in a particular cisterna interact to form structure too large to be included in transport vesicles. This model initially arose from an experiment demonstrating that oligomerization of a VSVG protein tagged with a *cis* Golgi localization signal correlated with the protein's retention (Weisz *et al.,* 1993). It has been further suggested that the immobility of these enzyme oligomers could be augmented by their binding to a putative Golgi matrix located among the cisternae (Slusarewicz *et al.,* 1994). In contrast, the second model proposes that the bilayer of the Golgi cisternae is not homogeneous but contains distinct lipid domains between which the Golgi enzymes partition differentially. The length of the transmembrane domain is viewed as the crucial factor in sorting resident Golgi proteins, which have shorter transmembrane domains than those of the plasma membrane. This model arose from the observations that Golgi transmembrane domains do not contain any obvious distinguishing sequence motif, attempts to find key residues by mutagenesis have been unsuccessful, and mammalian Golgi enzyme transmembrane domains are on average five residues shorter than those of the plasma membrane proteins (Bretscher and Munro 1993, Munro 1998). At present, the only aspect that appears to be agreed upon with respect to the retention of Golgi resident proteins is the importance of the transmembrane domain.

Aside from retention, there is evidence that retrieval is a factor in localization of residents to the Golgi. A tyrosine-based sorting signal, for example, is used for retrieval of TGN38 from the plasma membrane (Bos *et al.,* 1993, Farquhar and Palade 2000). Furthermore, a number of studies indicate that proteins normally residing in the Golgi apparatus also recycle back to the ER, possibly as a quality control function. When COPII-dependent ER export was blocked upon introduction of a dominant negative mutant of Sar1p, Golgi components including TGN glycosyltransferases gradually accumulated (Storrie *et al.,* 1998). Other evidence for the recycling of Golgi proteins to the ER comes from a retrograde transport assay based on the capacity of the ER to retain misfolded proteins. In this assay, the ectodomain of the temperature-sensitive VSVG ts045 was fused to Golgi or plasma membrane targeting domains. At the permissive temperature of 32^{o}C, the chimerae correctly localized to the Golgi apparatus or plasma membrane. When cells were shifted to the nonpermissive temperature of 40^{o}C, Golgi fusion proteins, but no plasma membrane proteins, reaccumulated in the ER due to misfolding of the ectodomain. Recycling was not induced by misfolding of the fusion proteins within the Golgi apparatus since the chimerae exhibited a misfolded phenotype only after redistribution into the ER. These results indicated that the chimerae normally cycled between the Golgi and the ER despite their Golgi localization phenotype, and became misfolded and retained at 40^{o}C while passing through the ER (Cole *et al.,* 1998). It is interesting to note that, unlike the retrieval pathway for ER resident proteins, the Golgi recycling pathway appears to be COPI-independent (Girod *et al.,* 1999). The rationale for such cycling of Golgi components through the ER could be that recycling is important for monitoring the fidelity of proteins that spend most of their lifetime in distal secretory compartments. For Golgi enzymes in particular, it could periodically expose them to the folding machinery in the ER where they could undergo further modification/repair or

degradation (Lippincott-Schwartz and Zaal 2000).

Intra-Golgi Transport

The most controversial topic in the trafficking of biosynthetic and secretory cargo is intra-Golgi transport. Two opposing models, vesicular transport and cisternal maturation, have resulted in considerable polarization of the field (Figure 3). In the vesicular transport model, COPI vesicles bud from one cisterna and fuse with another, delivering newly synthesized cargo in a *cis* to *trans* direction. Cisternae are distinct, stable, pre-existing structures; therefore, the Golgi apparatus as a whole is stable. Newly synthesized cargo that escapes is retrieved by specialized COPI carriers, predicting a dual function for COPI vesicles (Storrie *et al.,* 2000). Evidence in support of this model includes: the identification of two populations of COPI vesicles (one containing anterograde cargo) *in vivo* (Orci *et al.,* 1997), incorporation of an engineered anterograde cargo marker into COPI vesicles in a GTP and ARF1-dependent manner (Nickel 1998, Malsam *et al.,* 1999), and the identification of "mega-vesicles" that could explain the rapid transport of intra-cisternal aggregates across the Golgi stack (Volchuk *et al.,* 2000). In the cisternal maturation model, cisternae carry newly synthesized cargo towards the cell surface. COPI vesicles transport resident proteins and lipids in a retrograde direction, exchanging the content of each cisterna and thus balancing the flow of newly synthesized cargo. Cisternae are transient; therefore, the Golgi apparatus as a whole exists in a state of flux (Storrie *et al.,* 2000). Current evidence in support of this model includes: transport through the Golgi of green algal scales seemingly too large to be included in standard vesicles (Melkonian *et al.,* 1991), "maturation" of ER-to-Golgi intermediates in a process that selects anterograde cargo away from retrograde cargo (Shima *et al.,* 1999, Stephens *et al.,* 2000), and the ability of COPI to mediate retrograde transport from the Golgi to the ER (Cosson and Letourner 1994).

A recent model proposed by Pelham and Rothman (2000), attempts to pacify the polarization by combining both views. COPI vesicles would percolate up and down the Golgi apparatus bidirectionally (Orci *et al.,* 2000), providing both the fast track through which cargo flows across the Golgi and the recycling required to retain resident proteins. Cisternal progression would provide a slower track for larger aggregates. A second population of COPI vesicles would operate in a retrograde manner to return ER resident proteins/machinery to the ER. Nevertheless, despite this attempt to bring the two views into accord, it is likely that advocates of either the vesicular transport model or the cisternal maturation model will remain polarized for many years.

Conclusions

Over the course of evolution, cells have developed ways to monitor and regulate their activities. The biosynthetic pathway plays a pivotal role in compartment communication. A number of new techniques and a synergistic combination of established methods have resolved many questions and provided insight into this pathway. For example, the ER translocon has been demonstrated to exhibit bidirectionality, facilitating "input" of nascent polypeptides to the biosynthetic pathway and "output" of misfolded proteins to the proteasome. Additionally, transport between the ER and Golgi apparatus has been shown to rely on tubular carriers as well as vesicles. Moreover, a COPI-independent pathway for periodic cycling of Golgi proteins back to the ER has been reported. Nevertheless, many issues remain unresolved, particularly the mechanisms of intra-Golgi transport and protein sorting into the regulated secretory pathway. Resolution is likely to depend on collaboration between experts in current methods and on the development of innovative techniques.

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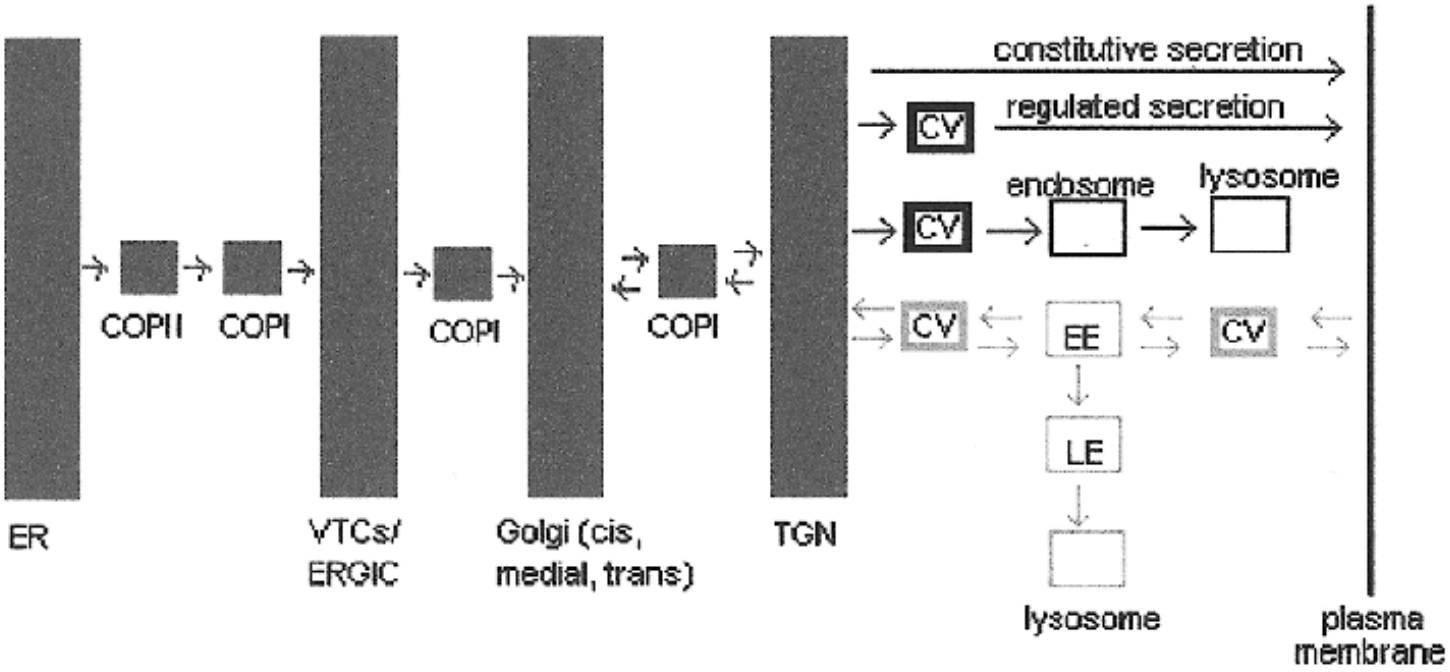

Figure 1. Schematic diagram of trafficking among the biosynthetic (filled), secretory (black), and endocytic (light gray) pathways. ER= endoplasmic reticulum, COPII= coatomer-like protein coat, COPI= coatomer protein coat, VTCs= vesicular-tubular clusters, ERGIC= endoplasmic reticulum-Golgi intermediate compartment, TGN= trans Golgi network, CV= clathrin-coated vesicle, EE= early endosome, LE= late endosome, thick rectangles represent coated carriers.

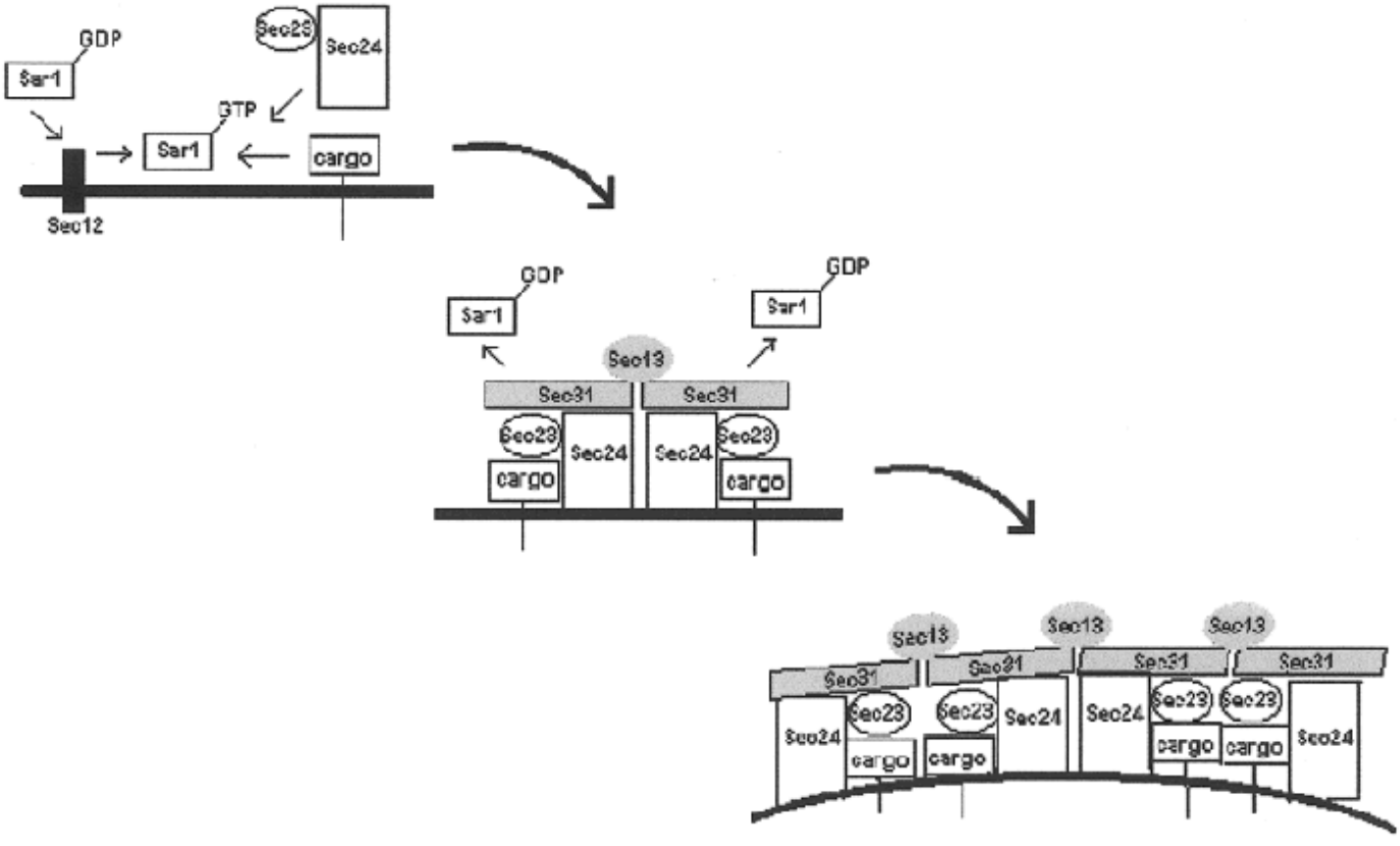

Figure 2. Model for COPII budding. Sec12p recruits Sar1p and facilitates exchange of GTP for GDP. Sar1p-GTP recruits Sec23p/Sec24p and cargo destined for packaging. Sec13p/Sec31p is then assembled onto the membrane and Sar1p hydrolyzes GTP before dissociating. Multiple cycles form a patch, a bud, and ultimately a COPII-coated vesicle. Note: Sec16p is also believed to participate in coat formation, but its role has not been thoroughly clarified.

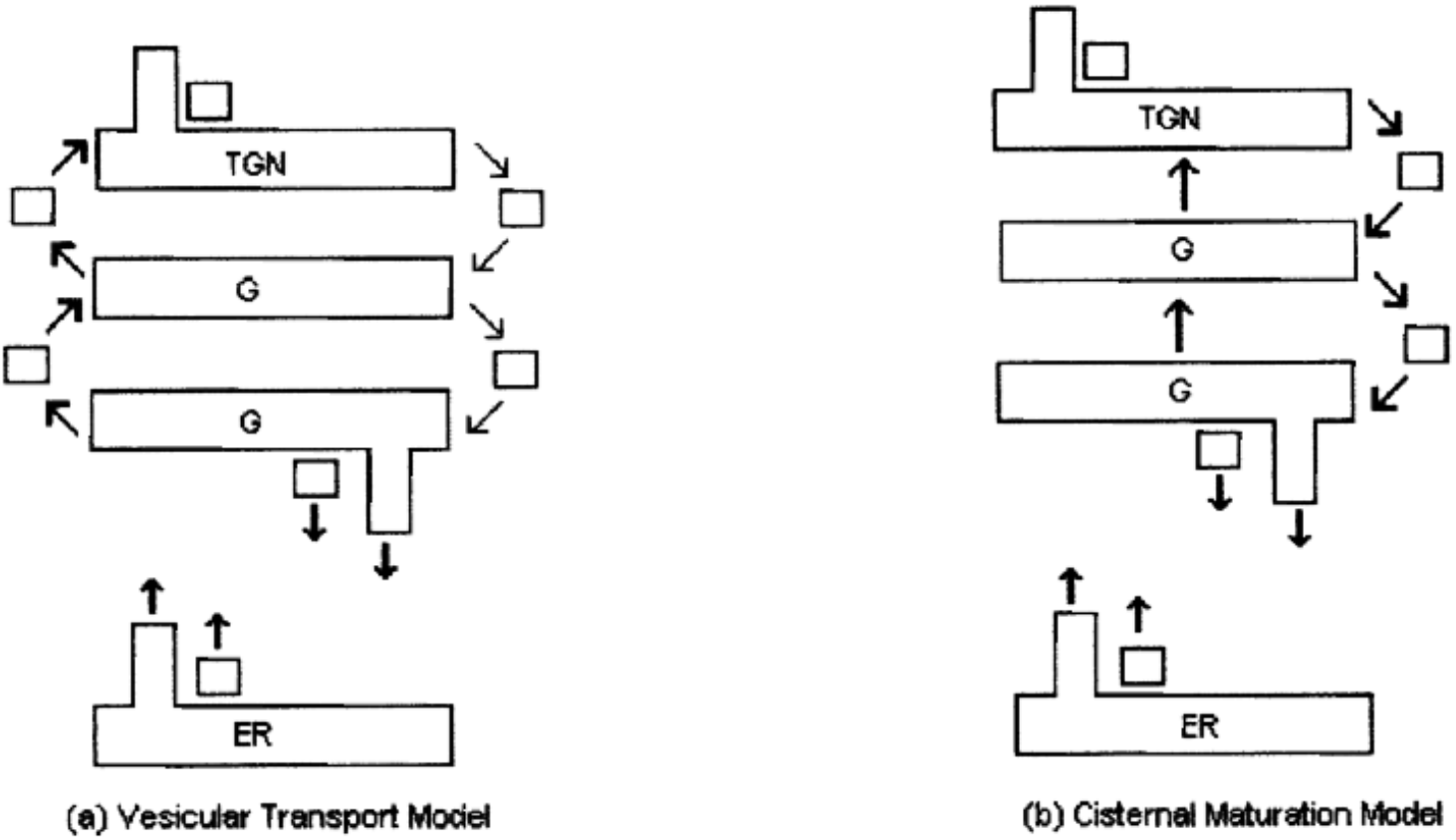

Figure 3. Vesicular transport and cisternal maturation models of intra-Golgi trafficking. (a) In the vesicular transport model, newly synthesized cargo is transported from cisterna to cisterna by COPI-coated vesicles. A low level of intra-Golgi retrograde transport by COPI-coated vesicles offsets leakage of resident components. (b) In the cisternal maturation model, the cisternae themselves are the cargo carriers and COPI-coated vesicles transport resident Golgi components in the retrograde direction from maturing cisternae.