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Sorting and Signaling at the Golgi Complex

Minireview

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The Golgi complex functions at the crossroads of the secretory pathway, receiving newly synthesized proteins and lipids from the ER, covalently modifying them, and then distributing the product to various final destinations (Figure 1). In addition, the Golgi recycles selected components back to the ER. The Golgi thus serves both as a processing station for newly synthesized glycoproteins and glycolipids derived from the endoplasmic reticulum (ER) and as a filtering system, separating proteins and lipids destined for the plasma membrane from those to be retained in the ER (for review see Mellman and Simons, 1992). To accomplish these functions, the Golgi is organized as polarized stacks (*cis* to *trans*) of flattened cisternae enriched in transmembrane processing enzymes. Selective trafficking and retention of protein and lipid species within this system is mediated by cytosolic coat proteins that assemble onto Golgi membranes, collect cargo, and help impart curvature to the lipid bilayer so budding of forward/retrograde transport intermediates can occur (Rothman and Wieland, 1996). Because transport intermediates that move into or out of the Golgi complex often travel significant distances through the cytoplasm, the Golgi is intimately associated with the cytoskeleton. In mammalian cells, the Golgi is centered at the microtubule organizing center and is actively maintained there by associations with microtubules and microtubule motors. An abundance of actin and actin binding proteins at the Golgi complex, surrounding the Golgi as a scaffold, may also facilitate the Golgi's spatial control of membrane traffic (De Matteis and Morrow, 1998).

How does the Golgi complex regulate cargo protein inclusion and vesicle budding, and coordinate these activities with the cytoskeleton to achieve spatial and temporal control of secretory traffic? Two recent studies (Goldberg, 2000; Wu et al., 2000), discussed in this review, stimulate new thinking in this area by suggesting that transmembrane cargo proteins and Cdc42, a Rho family GTPase that organizes actin, compete for similar motifs on the Golgi coatomer complex, COPI, which regulates secretory traffic. They thus provide a potential link between the mechanisms regulating membrane traffic and those controlling cytoskeletal organization.

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Golgi Coat Machinery and Its Role in Cargo Sorting

The heptameric cytosolic protein complex called COPI or coatomer, in conjunction with the GTP binding protein ARF1, forms an electron-dense coat that, when assembled onto Golgi membranes, is thought to facilitate membrane budding and fission events associated with Golgi membrane traffic (for review see Rothman and Wieland, 1996). Recruitment of COPI onto Golgi membranes requires ARF1, which, like all GTPases, cycles between a GDP-bound, inactive, and a GTP-bound, active, form. ARF1-GTP assembles COPI onto Golgi membranes, while GTP hydrolysis is thought to trigger membrane release of COPI into the cytosol, making COPI available for repeated cycles of coat assembly and disassembly. ARF1 thus operates as a binary switch to control COPI assembly onto membranes and thereby to regulate its function.

Originally, coatomer and ARF1 binding to membranes was thought to function exclusively and nonselectively in the formation of transport vesicles. This model assumed bulk flow of cargo through the secretory pathway and postulated that coatomer polymerization, driven by cycling of GTP on and off ARF1, provides the mechanico-chemical force for vesicle budding (for discussion see Mellman and Simons, 1992). A variety of findings since then has modified this view (Rothman and Wieland, 1996). ARF1 activation has significant effects on membrane phospholipid composition and stimulates

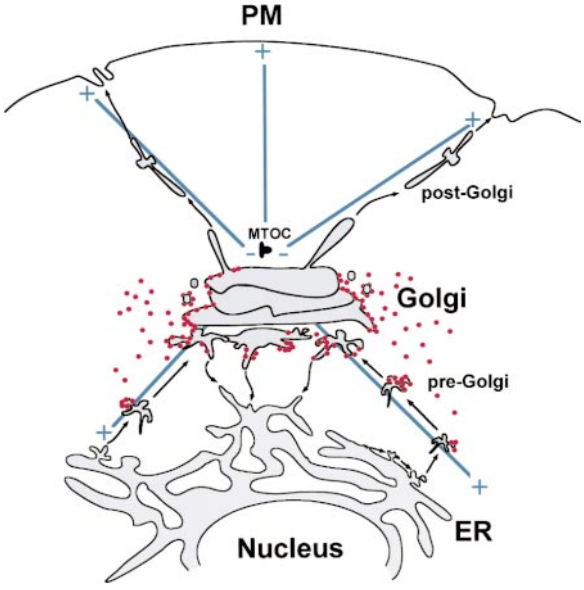

Figure 1. Diagram of the Secretory Pathway

Transport intermediates in the form of vesicles and tubulovesicular intermediates mediate forward and retrograde transport between the ER, Golgi complex, and plasma membrane (PM). They track plus- or minus-end directed along microtubules that emanate out of the microtubule organizing center (MTOC). Coatomer complexes (small dots) that cycle on and off Golgi and pre-Golgi membranes ensure selective trafficking of proteins within this system by collecting cargo and driving the formation of transport intermediates.

the assembly of spectrin, actin, and other cytosolic proteins onto Golgi membranes (De Matteis and Morrow, 1998). This suggests ARF1 could faciliate membrane sorting, budding, and docking processes at the Golgi complex. Coatomer, for its part, has been found to bind a pair of lysine residues in the C-terminal KKXX motif of transmembrane proteins that cycle between the Golgi and ER and which are thought to function as ER retrieval sequences (for review see Gaynor et al., 1998). By interacting with cytoplasmic tails of cargo proteins in this manner, COPI could gather up cargo into transport vesicles and mediate cargo sorting.

With regard to this latter function, it has been a point of major controversy whether membrane-bound COPI gathers cargo into coated vesicles that are retrograde-directed, forward-directed, or both. In yeast, mutant COPI subunits were identified in a screen designed to detect mutants unable to retain/recycle dilysine-tagged reporter molecules, but which still engage in forward traffic (see Gaynor et al., 1998 review). This suggested that COPI association with transmembrane cargo molecules containing dilysine motifs mediates their retrograde traffic. However, further analysis of certain *sec21* alleles (γ-COP) revealed cargo-selective defects in forward traffic as well (Gaynor et al., 1998). Furthermore, coatomer also recognizes related dilysine and diarginine sequences on the cytoplasmic tails of p24 proteins, a large family of putative cargo receptors abundant in the Golgi, that have been shown to engage in bidirectional transport (for review see Nickel and Wieland, 1998). These findings, together with previous biochemical and morphological data suggesting a role for coatomer in forward transport, make it unclear which direction (i.e., forward or retrograde) COPI-coated vesicles are transported. An additional possibility is that ARF1-mediated COPI association with membrane may serve to laterally segregate proteins and lipids into separate domains that later bud off as forward or retrograde transport intermediates. This function is suggested by the observations that blocking COPI membrane association with ARF1 inhibitory mutants or by brefeldin A (BFA) treatment (which prevents ARF activation) does not inhibit membrane traffic per se but deregulates it, resulting in proteins nonselectively entering a retrograde pathway back to the ER (Lippincott-Schwartz et al., 1998).

Signal Decoding by COPI

The recent work of Goldberg (2000) using an in vitro system with purified, soluble components has provided a powerful new approach for further defining the roles of coatomer, cargo, and ARF1 in the regulation of membrane traffic. His data suggest that association between COPI and cargo sorting sequences can itself influence ARF1 and COPI binding dynamics so that directional transport can be achieved. The ARF1 GTP binding and hydrolysis cycle, which underlies coatomer assembly onto membranes, is regulated by guanine nucleotide exchange factors (GEFs) that stimulate the exchange of GDP with GTP, and GTPase activating proteins (GAPs) that convert ARF-GTP to ARF-GDP. Last year, Goldberg (1999) published a crystal structure of the ARF GAP domain of ARF-GAP1 in complex with ARF1-GDP, which showed that the GAP domain made contacts with ARF1 at sites distant from the GTP binding pocket. This led him to investigate whether additional molecules such as COPI itself might participate in the ARF-bound GTP hydrolysis reaction, regulating return of ARF1 to its inactive, GDP-bound state. In an in vitro system with purified components and N-terminal truncated, nonmyristoylated ARF1, he found that COPI stimulates ARF GAP-catalyzed GTP hydrolysis on GTP-bound ARF1 (Goldberg, 1999). This suggested that after being recruited to membranes by ARF1, COPI modulates ARF activity, regulating the extent and lifetime of ARF1-GTP association with membranes.

Goldberg's recent work extends these findings to show that COPI's ability to modulate ARF1 activity is affected by cytoplasmic motifs on cargo molecules (Goldberg, 2000). He found that peptides derived from the cytoplasmic tails of a particular p24 protein, p24a, inhibited coatomer-stimulated ARF GAP activity, whereas peptides of other p24 proteins and dilysine retrieval sequences had no effect (Goldberg, 2000). Thus, COPI interaction with specific p24 cargo receptors or dibasic sequences in this system determines the lifetime of ARF-GTP and hence COPI association with membranes (assuming GTP hydrolysis on ARF is coupled to COPI release). The specificity for this response was quite remarkable in that p24a (FFEVRRVV) inhibited COP-stimulated GAP whereas p24d (FFEAKKLV) had no effect. Upon COPI interaction with p24a, therefore, GTP hydrolysis on ARF1 may be delayed, allowing coated vesicle formation to occur. COPI interaction with other p24 proteins or dilysine motifs, however, would result in rapid hydrolysis of ARF-bound GTP without time for coat assembly (Goldberg, 2000).

Goldberg's model of how COPI might decode sorting signals in this manner to specify "coating lifetime" is appealing, but needs to be tested under more physiological conditions. Recently, Szafer et al. (2000) confirmed Goldberg's results, showing that COPI stimulates ARF1-GAP catalyzed GTP hydrolysis of truncated ARF1. However, they found that the activity of ARF-GAP1 is significantly greater when phospholipid micelles and full-length, myristoylated ARF1 is used in the assay. More importantly, they showed that COPI had no effect on GAP-catalyzed GTP hydrolysis under these conditions. Although the use of phospholipid micelles and myristoylated ARF1 in Szafer et al.'s assay represents an attempt to resemble the conditions and site where ARF-GTP is hydrolyzed (i.e., the Golgi membrane surface), lipid micelles are not Golgi membranes. The discrepancy between Goldberg and Szafer et al.'s findings will need to be resolved using intact Golgi membranes that contain ARF-GEFs, -GAPs, and p24 proteins. Given the evidence that GTP hydrolysis on ARF is required for COPI-mediated protein sorting (Lanoix et al., 1999), a major challenge of future work in this area will be the development of new methods (both in vitro and in vivo) for monitoring cargo sorting reactions in Golgi membranes in the presence of ongoing cycles of nucleotide exchange and hydrolysis on ARF1.

Cdc42, a New Binding Partner of COPI

While Goldberg's findings are helping to clarify the role of COPI in the mechanism(s) of cargo inclusion and the regulation of vesicle budding, a recent study by Cerione and colleagues (Wu et al., 2000) provides new clues toward understanding how these processes might be coupled to signal transduction pathways controlling the

cytoskeleton. Cdc42, a Rho family GTPase that localizes to the Golgi complex, has been found to interact with the γ-COP subunit of coatomer via a dilysine motif present at the carboxyl terminus of Cdc42. This raises the possibility that Cdc42 could influence coatomer dynamics and Golgi function, or vice versa.

Cdc42 is known to control the organization of actin filaments and perhaps other cytoskeletal elements that direct transport of secretory vesicles (for reviews see van Aelst and D'Souza-Schorey, 1997 and Drubin and Nelson, 1996). For example, in yeast cells expressing defective *cdc42* alleles, growth is nonpolarized instead of bud-directed. Moreover, T cells expressing Cdc42 mutants are unable to reorient their MTOC and Golgi complex in response to binding to an antigen presenting cell, while epithelial cells expressing these mutants show a selective loss of basolateral polarity (Drubin and Nelson, 1996; Kroschewski et al., 1999). Additionally, Cdc42 mediates signaling pathways that regulate cell growth control and transformation (van Aelst and D'Souza-Schorey, 1997). With these observations in mind, Cerione and colleagues searched for new effector proteins of Cdc42 in order to understand how Golgi-localized Cdc42 might coordinate such activities from this central location. They focused on binding partners that are required for the cellular transformation phenotype found with a rapid cycling mutant of Cdc42, F28L, which exchanges nucleotide more readily and hence spends more time in the GTP-bound form. A similar transformation phenotype is observed in cells expressing Dbl, an exchange factor for Rho GTPases.

During their search, Cerione and colleagues identified the γ-COP subunit of coatomer as a protein that binds the GTP-bound form of Cdc42 via a dilysine (KK) motif present at the carboxyl terminus of Cdc42 (Wu et al., 2000). The ability of Cdc42/F28L to transform 3T3 cells was dependent upon the KK motif, as mutation of these residues inhibited transformation, and expression of the N-terminal portion of γ-COP that can bind KK, but not associate with other COPI subunits, blocked Cdc42-induced but not Ras-induced transformation (Wu et al, 2000). In addition to inducing transformation, expression of Cdc42/F28L resulted in a 1.5- to 2-fold stimulation in the rate of secretory protein transport, as measured by acquisition of carbohydrate modification after release of the vesicular stomatis viral G protein from the ER. This effect on secretion was also dependent upon the KK motif, but separable from full induction of a transformed phenotype, since deletion of the Rho insert region in Cdc42/F28L (a region previously shown to be required for Cdc42 transformation) still exerted a stimulatory effect on secretion (Wu et al., 2000).

The extent to which these findings can be generalized requires further investigation. The dilysine motif found in Cdc42, a peripheral membrane protein, is not typical in sequence or positioning to that found on the cytoplasmic tails of type I transmembrane proteins (-KKXX) of the early secretory pathway. Furthermore, the positional context of the KKXX ER retrieval signal is known to be critical for COPI binding (e.g., KKXXX is nonfunctional) (Nickel and Wieland, 1998). Fully processed Cdc42 (-KKSRRC) is also lipid modified on the cysteine at the C terminus, a modification required for biological activity. The fact that all Rho proteins have a series of basic amino acids adjacent to the C terminus, believed to be necessary in combination with the lipid moiety for tight membrane association, therefore, raises some questions as to the specificity of this interaction, although Wu et al. only saw binding to COPI by Cdc42 and not by other Rho family members. Finally, the stimulation of secretion induced by expression of the fast-cycling mutant F28L is a modest one, so how it might be necessary for transformation, if at all, is not clear. Despite their preliminary nature, Cerione's findings are provocative because they point to a mechanism whereby the Golgi could coordinate the regulation of membrane traffic, cytoskeleton organization, and signal transduction.

p24 Proteins and Cdc42: Competing for COPI Binding?

The observations from both Goldberg's and Cerione's labs hint at the possibility of a complex interplay between ARF, its GAP, COPI, the p24 proteins, and Cdc42 activation at the Golgi complex. The mechanism whereby Cdc42/F28L stimulates secretion is not known, but could, given Goldberg's findings and the observation that Cdc42 interacts with COPI, be mediated by an alteration in ARF/COP dynamics. For example, activated Cdc42 could compete with sorting signals on cargo proteins for binding to coatomer, causing modulation of ARF GAP activity and exclusion or inclusion of cargo into transport vesicles, and thereby stimulate secretion. The apparent stimulation of secretion by Cdc42/F28L shown by Wu et al. (2000) may be related to the observation of Kroschewski et al. (2000), who demonstrated a requirement for Cdc42 in polarized transport from the Golgi in MDCK cells. Testing these hypotheses using a wide variety of experimental systems (including in vitro and whole cell assays) will be important to pursue. Such systems need to address whether signal transduction pathways that activate Cdc42 affect associations between COPI and cargo to alter secretory transport, and whether other signaling molecules (i.e., phosphoinositides and heterotrimeric G proteins) modulate secretory trafficking in an analogous manner. As one example, stimulation of receptors that activate protein kinase C (PKC) has been shown to enhance constitutive secretion and to affect binding of ARF1 and COPI to Golgi membranes. The recent finding that activated PKCε is recruited to the Golgi complex through binding to β′-COP (Csukai et al., 1997) suggests that PKCε may be the isoform responsible for this activity.

How enhanced Cdc42–COPI interactions mediate transformation, as suggested in the Wu et al. study, is unclear. COPI is unlikely to participate directly in cell transformation, which is a complex series of events that includes activation of transcription factors and various kinase pathways. A more attractive hypothesis is that association with coatomer makes Cdc42 a more effective signaling molecule. In this model, coatomer would behave as an adaptor molecule to facilitate Cdc42's signaling functions. There are several reasons why association with coatomer might make Cdc42 a more effective signaling molecule. First, by associating with coatomer, Cdc42 is brought to regions of the membrane containing activated ARF1, which is known to alter lipid composition of membranes by activation of phospholipase D (PLD) and by recruitment and activation of phosphatidylinositolphosphate (PIP) kinases leading to production of polyphosphatidylinositol 4,5-bisphosphate

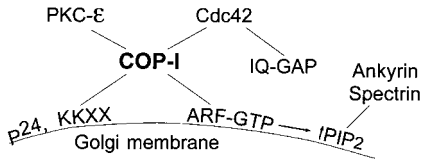

Figure 2. COPI Interacting Proteins at the Golgi Complex

COPI is recruited to Golgi membranes by ARF1-GTP. COPI can interact with a variety of Golgi-associated proteins as indicated by connecting lines including cytoplasmic tails of transmembrane proteins (KKXX and p24 proteins), Cdc42-GTP, and PKCε. ARF1-GTP also stimulates the production of PIP_2 on Golgi membranes leading to the recruitment of spectrin and ankyrin.

(PIP_2) (Godi et al., 1999). Since PIP_2 has been shown to stimulate nucleotide exchange in the absence of GEF (Zheng et al., 1996), local PIP_2 production could further enhance the GTP loading of Cdc42. Second, since COPI association occurs at the carboxyl terminus of Cdc42, the Ras effector loop and Rho insert region on Cdc42 are available for interaction with effector functions. One such effector could be IQGAP, an actin binding protein and putative Cdc42 effector, that has been localized to the Golgi complex and is associated with Cdc42 (McCallum et al., 1998).

The Golgi as a Signaling Platform

The findings that coatomer and Cdc42 interact at the Golgi complex with effects on both secretory traffic and cellular growth control raises the possibility that the Golgi serves as a platform for membrane sorting and cell signaling. Many features of the Golgi complex make it an ideal location for the regulation of membrane traffic and signal transduction. It is situated between the ER and plasma membrane, intersecting a variety of membrane trafficking pathways. In addition, its membrane composition, intermediate between the ER and cell surface and enriched in phosphoinositides (known for their role in signal transduction), is ideal for producing changes in lipid composition which might affect the activity of signaling molecules and/or components of trafficking machinery. Consistent with this, a variety of signaling molecules associate with Golgi membranes, including heterotrimeric G proteins, PI(3)kinase, eNOS, and Cdc42 (Stow and Heimann, 1998; Godi et al., 1999; Sowa et al., 1999; and Wu et al., 2000). Golgi membranes also interact with a variety of motor and cytoskeletal proteins, including p200/myosin II, myosin I, V, and VI, dynein, spectrin, and ankyrin, that facilitate the Golgi's spatial control of membrane traffic but also might help to coordinate signaling pathways (De Matteis and Morrow, 1998).

How might such a platform be organized and regulated? It has been known for several years that a membrane skeleton, whose components include the actin binding proteins spectrin and ankyrin, associates with the cytoplasmic surface of the Golgi. This scaffold, together with other peripherally associated Golgi proteins (including many of the signaling molecules above) is perturbed acutely by BFA treatment. This suggests that their association with the Golgi is either directly dependent upon ARF1-GTP or dependent upon a scaffold whose assembly is initiated by ARF1 activity. Recently, it has been shown that ARF1 activity increases $PtdIns(4,5)P_2$ levels in the Golgi through the recruitment of PI4Kβ to Golgi membranes (Godi et al., 1999). Since PIP_2 generation leads to the assembly of actin and spectrin on Golgi membranes (De Matteis and Morrow, 1998), other molecules could become associated and stabilized in this area, possibly enhancing communication with other signaling molecules and microtubules (see Figure 2). In this way, PIP_2 generation by ARF1 could serve a variety of related signaling functions by recruiting molecules to the membrane, modulating the activities of ARF regulatory molecules, and as a cofactor for ARF-stimulated PLD (Godi et al., 1999). Researchers in the fields of membrane trafficking, cytoskeleton and signal transduction now need to collaborate in detailing the role of these regulatory molecules, together with Cdc42 and COPI, in protein sorting and signaling from the Golgi complex.

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