Mechanisms of transport through the Golgi complex

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Summary

The Golgi complex is the central sorting and processing station of the secretory pathway, ensuring that cargo proteins, which are synthesized in the endoplasmic reticulum, are properly glycosylated and packaged into carriers for transport to their final destinations. Two recent studies highlight the fact that properties of membrane lipids play key roles in Golgi structural organization and trafficking. The Antonny laboratory has demonstrated the mechanism by which a Golgi tether containing a membrane-curvature-sensing domain at one end can link highly curved and flat membranes together in a reversible manner. In this way, a strong interaction that binds membranes together in an oriented fashion can easily be disrupted as the properties of the membranes change. The Lippincott-Schwartz laboratory has developed a new model for intra-Golgi trafficking, called the rapid-partitioning model, which incorporates lipid trafficking as an integral part. Simulations reveal that the sorting of lipids into processing and export domains that are connected to each Golgi cisterna, and bidirectional trafficking throughout the Golgi to allow proteins to associate with their preferred lipid environment, is sufficient to drive protein transport through the secretory pathway. Although only a proof in principle, this model for the first time invokes lipid sorting as the driving force in intra-Golgi trafficking, and provides a framework for future experimental work.

Key words: Golgi, Secretory pathway, Intra-Golgi trafficking, Membrane curvature, ALPS motif, Tether, Golgin, Sphingolipid, Glycerophospholipid, ADP ribosylation factor, G protein, COPI, Coatomer, Glycosylation

Introduction

Because of its structural complexity and highly dynamic nature, understanding the mechanisms that regulate trafficking through the Golgi complex has been particularly elusive. In the secretory pathway, this organelle assures the post-translational modification of secretory cargo proteins by processing enzymes (e.g. glycosyltransferases, sialyltransferases) and therefore must coordinate forward cargo movement with an assembly line of processing enzymes that sequentially modify the cargo proteins (Dean, 1999; Helenius and Aebi, 2001; Lowe and Marth, 2003; Opat et al., 2001). The Golgi complex in mammalian cells consists of stacks of cisternae that are connected by tubulovesicular regions (Mogelsvang et al., 2004; Morré and Mollenhauer, 2007; Rambourg and Clermont, 1990). These have been referred to as 'compact' and 'non-compact' zones, respectively, and they are linked together to form a continuum, the Golgi ribbon (Fig. 1). Golgi processing enzymes that act early are concentrated in *cis*-Golgi saccules, whereas those that act later are found mostly in the *trans*-Golgi (Farquhar and Palade, 1981; Kweon et al., 2004; Martinez-Menarguez et al., 2001). In view of this gradient of successively acting glycosylation and other processing enzymes, it has generally been thought that the cisternae are the important functional units of the Golgi that cargo passes through so as to receive the ordered series of glycosylations that are required for maturation. However, processing enzymes are also found in the tubular non-compact zones of the Golgi (Kweon et al., 2004; Martinez-Menarguez et al., 2001).

In fungi and plants, the Golgi is not organized as a continuous ribbon, but is made up of distinct units that are scattered throughout the cytoplasm (Fig. 2). In plants and some yeasts, these units are considered to be made up of a series of stacked cisternae, with surrounding tubular and vesicular profiles (Kepes et al., 2005; Mogelsvang et al., 2003). However, in other yeasts, such as *Saccharomyces cerevisiae*, there are few if any cisternae, and the

Golgi units are made up of tubular networks (Kepes et al., 2005; Matsuura-Tokita et al., 2006; Rambourg et al., 2001). Given the high conservation from yeast to humans of proteins that regulate transport through the Golgi complex (Behnia and Munro, 2005; Lee et al., 2004), it is likely that tubular networks have a fundamental role in the secretory pathway (Morré and Mollenhauer, 2007).

Many membrane-trafficking steps occur via the budding of small transport vesicles from a donor compartment, followed by targeting to and fusion with an acceptor compartment (Fig. 3A). However, some trafficking steps employ larger carriers, such as Golgi-to-plasma-membrane (PM) transport, which is assured by large pleiomorphic structures (Luini et al., 2005; Polishchuk et al., 2003). The different stages of the vesicle budding and fusion process can be reconstituted in vitro, a powerful approach that has led to the assignment of specific functions for proteins that are involved in each step of a transport cycle (Bonifacino and Glick, 2004; Gillingham and Munro, 2007; Lee et al., 2004). Coat proteins, large multi-subunit complexes that cycle between the membrane and cytosol, are required for vesicle budding (Bonifacino and Glick, 2004; Gurkan et al., 2006). Coat proteins are recruited to membranes from the cytosol by a small G protein in its active GTP-bound form, where they mediate both membrane deformation and protein sorting. After scission from the donor membrane, the vesicle is then associated with its target membrane through one or more tethering proteins, which function to link two membranes together (Behnia and Munro, 2005; Short et al., 2005). Once docked in this way to the appropriate acceptor membrane, fusion – mediated by SNARE proteins – ensues (Jahn, 2008) (Fig. 3A).

How the well-studied processes of vesicle formation and fusion are integrated into an overall framework for understanding intra-Golgi transport is still the subject of considerable debate. An important feature of this process is the fact that the Golgi must maintain its structural integrity in the face of an incessant, sometimes
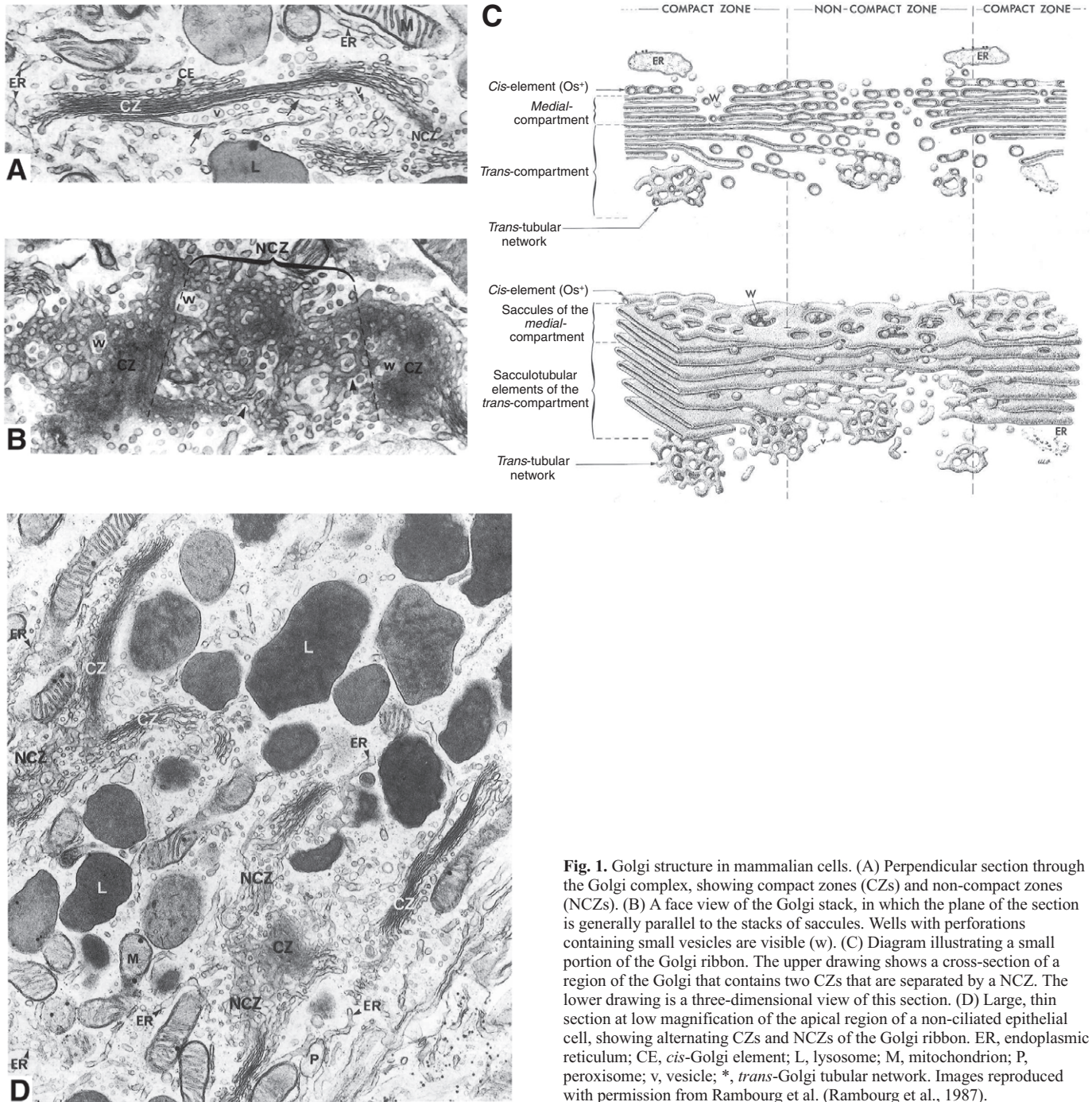
Fig. 1. Golgi structure in mammalian cells. (A) Perpendicular section through the Golgi complex, showing compact zones (CZs) and non-compact zones (NCZs). (B) A face view of the Golgi stack, in which the plane of the section is generally parallel to the stacks of saccules. Wells with perforations containing small vesicles are visible (w). (C) Diagram illustrating a small portion of the Golgi ribbon. The upper drawing shows a cross-section of a region of the Golgi that contains two CZs that are separated by a NCZ. The lower drawing is a three-dimensional view of this section. (D) Large, thin section at low magnification of the apical region of a non-ciliated epithelial cell, showing alternating CZs and NCZs of the Golgi ribbon. ER, endoplasmic reticulum; CE, *cis*-Golgi element; L, lysosome; M, mitochondrion; P, peroxisome; v, vesicle; *, *trans*-Golgi tubular network. Images reproduced with permission from Rambourg et al. (Rambourg et al., 1987).

massive, flow of material passing through it. As with many biological systems, the concept of self-organization is important in formulating a model for intra-Golgi trafficking. Two recent papers, highlighted in this Commentary, provide novel insights into the mechanisms of Golgi self-organization (Drin et al., 2008; Patterson et al., 2008). Drin et al. used sophisticated biophysical approaches in a highly purified system to fully dissect the mechanism by which a Golgi tether reversibly binds two membranes together. This study demonstrates the mechanism by which an important Golgi structural component can function in a highly dynamic manner to adapt to changes in the underlying membrane. The Patterson et al. study used kinetic analysis of cargo trafficking in intact cells and modeling

to develop a new paradigm for transport through the Golgi complex. This new model for intra-Golgi transport incorporates lipid trafficking as an integral part, with lipid sorting proposed to occur at each level of the Golgi stack to drive transport through the organelle. Both studies emphasize the importance of lipids in the self-organization of the Golgi complex.

The Golgi as a self-organizing structure

There are three distinct mechanisms by which cellular structures can be formed (Karsenti, 2008; Kirschner et al., 2000; Misteli, 2001). The first is template driven, in which a new structure is produced using a pre-existing structure as a model. The DNA double
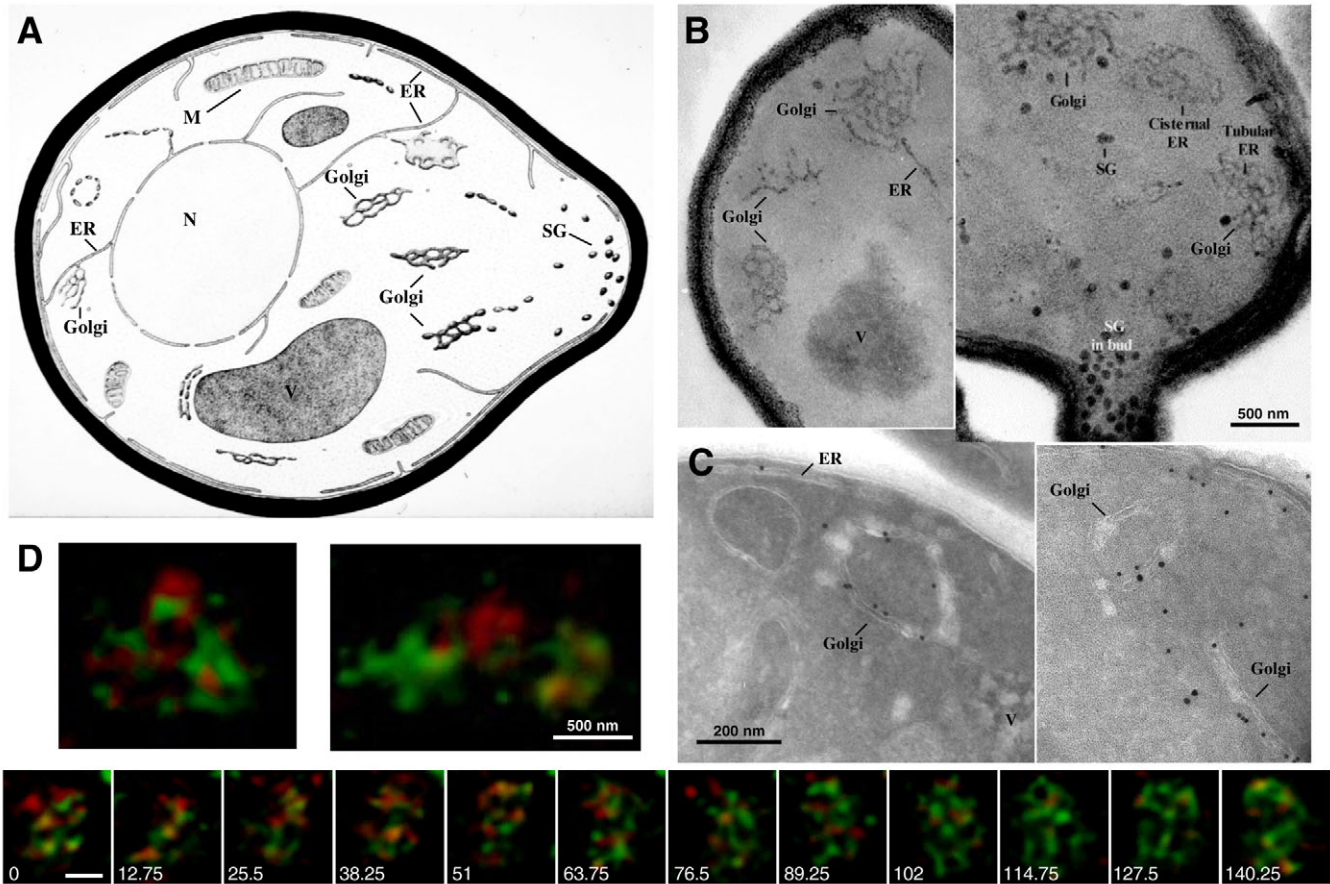
Fig. 2. Yeast Golgi morphology. (A) Diagram of intracellular organelles of the yeast *Saccharomyces cerevisiae*, summarizing the different types of membrane structures observed by electron microscopy (EM). V, vacuole; M, mitochondrion; ER, endoplasmic reticulum; SG, secretory granules or vesicles; Golgi, Golgi element; N, nucleus. (B) Thin-section electron micrographs showing membrane structures in *S. cerevisiae*. (C) Immuno-EM staining of yeast Golgi elements. Left panel: Och1-HA labeled with 10-nm gold (early Golgi). Right panel: double-labeling Och1-HA, 10 nm gold (*cis*-Golgi) and Kex2p, 5 nm gold (TGN). (D) Confocal light-microscopy images of yeast Golgi marked with two different fluorescent markers: red, mRFP-Gos1p (*medial*-Golgi); green, Sec7-GFP (*trans*-Golgi). Bottom panels: time-course observation (seconds) of a Golgi element expressing mRFP-Gos1p and Sec7-GFP, as above. Scale bar: 500 nm. Images in D reproduced with permission from Matsuura-Tokita et al. (Matsuura-Tokita et al., 2006).

helix is an example of this type of structure. The second mechanism is self-assembly, in which interactions between the component parts of a structure spontaneously lead to its formation. At equilibrium, the final product is stable. Viral capsids are formed by this type of process. The third mechanism is self-organization, which, similar to self-assembly, is driven by interactions between the component parts and not by external forces or templates. However, at steady state, a self-organizing system is far from achieving equilibrium, and a constant supply of energy is required to maintain it. If energy input into the system is withdrawn, the structure rapidly disappears (Karsenti, 2008). Examples of self-organized structures are microtubules, which exhibit dynamic instability, as well as entire microtubule-based systems such as the mitotic spindle (Karsenti, 2008; Kirschner et al., 2000).

The Golgi complex is another example of a self-organizing structure (Misteli, 2001). Studies of the effect of the drug brefeldin A (BFA) on the Golgi were the first to reveal the self-organizing properties of this organelle (Klausner et al., 1992). BFA, a highly specific drug that has a well-understood molecular mechanism, blocks activation of the small G protein ADP ribosylation factor 1 (Arf1), which cycles between inactive GDP-bound and active GTP-bound states (Mossessova et al., 2003; Peyroche et al., 1999; Renault

et al., 2003). Guanine nucleotide-exchange factors (GEFs) are required to catalyze the exchange of GDP for GTP on Arf proteins, and GTPase-activating proteins (GAPs) return the activated form to its GDP-bound state by hydrolysis of GTP (Gillingham and Munro, 2007). BFA is an uncompetitive inhibitor of the exchange reaction: it binds to a GEF-Arf1-GDP reaction intermediate, thus blocking Arf1 activation (Peyroche et al., 1999; Robineau et al., 2000). When cells are treated with BFA, the entire Golgi complex completely disassembles within minutes, with much of its membrane becoming adsorbed into the endoplasmic reticulum (ER) (Sciaky et al., 1997). When BFA is washed out, the Golgi reforms and returns to its steady-state structure. These results indicate that a constant input of energy through cycles of GTP binding and hydrolysis on Arf1 are required to maintain Golgi structure, a hallmark of a self-organizing system.

These data raise the important issue of how Arf1 maintains Golgi structure. This task is assured by the effectors that bind specifically to active Arf1-GTP, including coat complexes [such as coatomer complex protein I (COPI); Golgi-localized, γ-ear-containing, Arf-binding proteins (GGA)-clathrin; and adaptor protein 1 (AP-1)-clathrin], lipid-modifying enzymes [such as phosphotidylinositol 4-kinase (PI4K) and lipid-transfer proteins] and membrane tethers
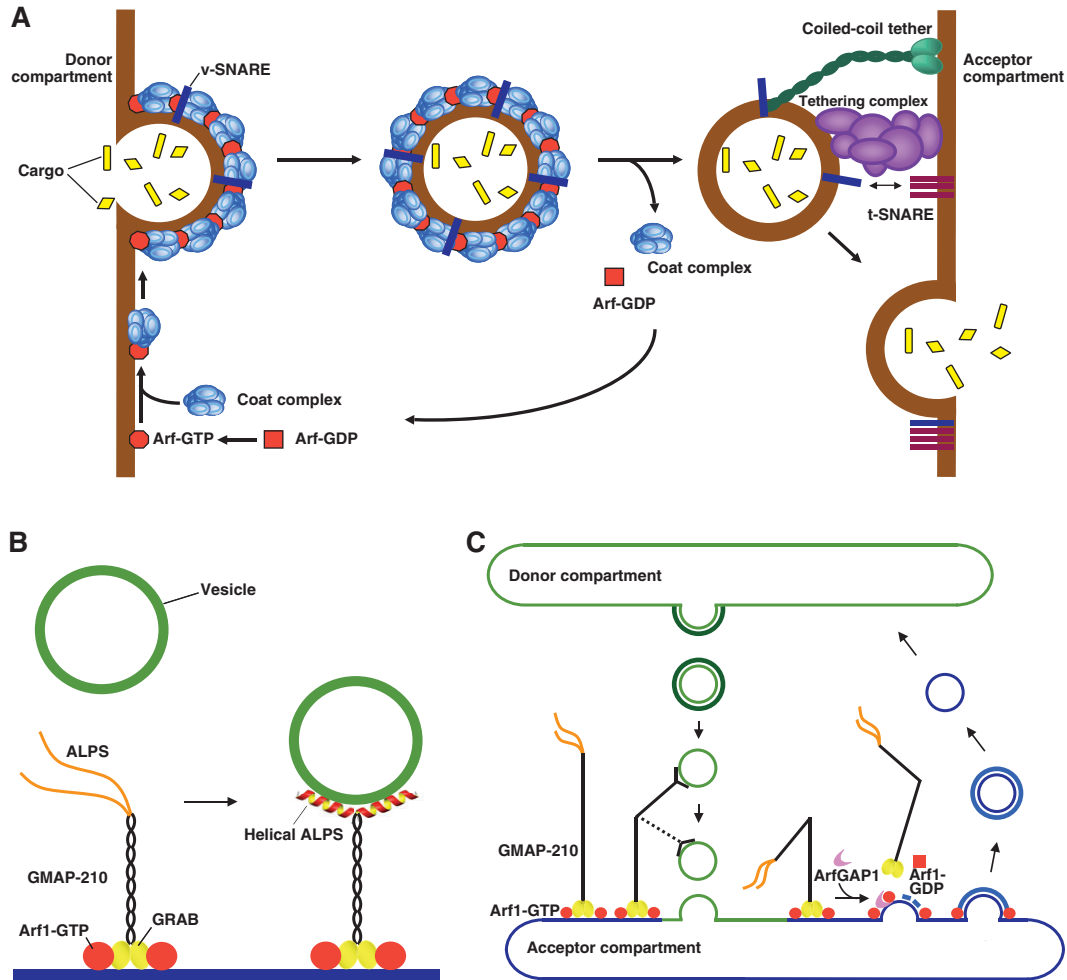
Fig. 3. (A) Vesicle budding and fusion. Activation of a small G protein (such as Arf1 or Sar1) (red) by the exchange of GDP for GTP results in the recruitment of a coat complex (blue) to the membrane by the GTP-bound form of the G protein. Membrane curvature and sorting of cargo (yellow) into the forming bud ensue, followed by fission of the coated bud to form a vesicle. Hydrolysis of GTP eventually leads to release of the coat from the vesicle. The vesicle is targeted to the acceptor-compartment membrane by tethering complexes (long coiled-coil, green; multi-subunit, purple). For simplicity, the coat is not shown at this stage, but note that the coat may in some cases remain on the vesicle during the tethering process, with uncoating occurring after tethering. The vesicle SNARE (v-SNARE; dark blue bars) on the vesicle engages the tripartite target-localized SNARE (t-SNARE; maroon bars), which leads to fusion of the vesicle and acceptor-compartment membranes, and to the release of cargo into the acceptor compartment. (B) Tethering of highly curved and flat membranes by GMAP-210. The C-terminus of GMAP-210 contains a GRAB domain (yellow) that binds to membranes containing Arf1-GTP (red circles). In the absence of highly curved membranes, the N-terminal ALPS motif (orange) of GMAP-210 is unstructured. When the ALPS motif comes into contact with a highly curved membrane such as a vesicle (green), it folds into an amphipathic helix that binds tightly to the vesicle membrane. Note that the C-terminal GRAB domain of GMAP-210 binds only to flat membranes, owing to curvature-stimulated ArfGAP1 activity on Arf1-GTP. (C) Model for the role of GMAP-210 in cells. A donor compartment (green; top) produces coated vesicles that are directed towards an acceptor compartment (dark blue; bottom). GMAP-210, via its GRAB domain (yellow), binds to Arf1-GTP (red circles) on the flat membrane of the acceptor compartment. The ALPS motif (orange) of GMAP-210 binds to the incoming vesicle (shown here after uncoating) and directs it to the fusion site of the acceptor-compartment membrane. After fusion, the loss of curvature in the vesicle membrane results in release from the GMAP-210 ALPS motif. Arf1-GTP is involved in the sorting of components that are to be recycled back to the donor compartment through formation of retrograde transport vesicles. When these vesicles begin to form on the acceptor-compartment membrane, hydrolysis of GTP on Arf1 by ArfGAP1 (pink) at regions of positive curvature in the membrane of the budding vesicle results in release of the GMAP-210 GRAB domain from the membrane.

(Behnia and Munro, 2005; Bonifacino and Glick, 2004; Gillingham and Munro, 2007). The lipid-transfer proteins four-phosphate adaptor protein 2 (FAPP2; also known as PLEKHA8), and possibly oxysterol-binding protein 1 (OSBP) and ceramide transfer protein (CERT), are Arf1 effectors that extract lipid precursors from the ER and transfer them directly to Golgi membranes (De Matteis and Luini, 2008). The most recently identified class of Arf1 effectors are tethers, including the long coiled-coil protein Golgi microtubule-associated protein of 210 kDa (GMAP-210; also known as thyroid receptor-interacting protein 11, TRIP-11) (Drin et al., 2008; Gillingham et al., 2004). The identification of a tether as an Arf1

effector might seem surprising at first glance, as Arf1 is well known for its function in vesicle budding. However, it makes sense that vesicle targeting components should be put in place at the time of carrier-vesicle formation to assure correct delivery of the vesicle to its appropriate acceptor compartment (Allan et al., 2000; Cai et al., 2007; Short et al., 2005).

A new class of Arf1 effectors – Golgi tethers
There are two major classes of tethers, one consisting of large, multisubunit complexes such as transport protein particle (TRAPP) and the exocyst, the other consisting of long coiled-coil proteins

(Cai et al., 2007; Short et al., 2005; Whyte and Munro, 2002). The Arf1 effector GMAP-210 is a member of the latter class. Originally identified by Rios, Bornens and colleagues, this protein localizes to the Golgi and is involved in Golgi organization (Infante et al., 1999; Rios et al., 2004). Strikingly, overexpression of GMAP-210 in mammalian cells causes the accumulation of large aggregates of small vesicles that are 50 nm in diameter (Pernet-Gallay et al., 2002). The Munro laboratory made the key discovery that, in its C-terminal region, GMAP-210 contains a GRAB (GRIP-related Arf binding) domain that specifically binds Arf1-GTP and is highly conserved in evolution (Gillingham et al., 2004). A related domain, the GRIP domain, which is found in the C-terminal regions of a number of long coiled-coil proteins, had been previously identified as an effector of the Arf-related GTPase Arl1 (Gillingham and Munro, 2007; Short et al., 2005). Hence, GMAP-210 belongs to a larger family of long coiled-coil proteins that bind to an Arf-family GTPase at their C-terminus. Two different tethers, p115 (also known as TAP) and CASP, were found to bind to distinct sub-populations of COPI vesicles, demonstrating that tethers confer specificity by linking a specific type of vesicle to a given acceptor compartment (Malsam et al., 2005). In the case of GMAP-210, the C-terminal portion binds to membranes in an Arf1-GTP-dependent manner, but what about the other end? The discovery of an ALPS (amphipathic lipid-packing sensor) motif at the N-terminus of GMAP-210 provided the first clue.

Membrane-curvature sensing as a driving force in Golgi organization

The ALPS motif is a membrane-binding module that is highly sensitive to membrane curvature (Bigay et al., 2005; Drin et al., 2007). ALPS motifs bind with high affinity to liposomes that are less than 100 nm in diameter, but with much lower affinity to liposomes that have a diameter greater than 200 nm. ALPS motifs are unstructured in the absence of a lipid bilayer, but are induced to form an amphipathic helix when they come into contact with a highly curved membrane (Drin et al., 2007) (Fig. 3B). In this way, they are membrane-curvature sensors. The first ALPS motifs were identified in ArfGAP1, which efficiently hydrolyzes GTP on Arf1 that is bound to small-diameter liposomes, but is practically inactive on large liposomes (Bigay et al., 2005). Subsequently, a bioinformatics search revealed the presence of ALPS motifs in a wide range of proteins that are involved in membrane processes, including nuclear-envelope-binding components of the nuclear pore, proteins involved in ER-Golgi trafficking and lipid-transfer proteins (Drin et al., 2007).

The presence of an ALPS motif at the N-terminus of GMAP-210 and a GRAB domain at its C-terminus suggested that this tether links highly curved membranes to flat membranes (Fig. 3B). Using dynamic-light-scattering analysis and both fluorescence and electron microscopy (EM), Drin et al. carried out experiments that proved this intriguing hypothesis (Drin et al., 2008). Binding of the Arf1-GTP-specific GRAB domain exclusively to membranes of low curvature is achieved by the action of ArfGAP1, which, through its own ALPS motifs, catalyzes the hydrolysis of GTP on Arf1 only on highly curved membranes (Fig. 3B,C). The vectorial nature of the tethering interaction was shown by using a mixture of large liposomes coated with Arf1-GTP and small naked liposomes, which could form large aggregates upon the addition of GMAP-210, but only if this specific configuration of components was used. EM analysis indicated that the large liposomes were encircled by small ones, which often formed chains along the surface of the large liposomes. In a final series of experiments, Antonny and colleagues developed a system

amenable to light microscopy using giant liposomes labeled with a green fluorophore and small liposomes labeled with a red one. Along with Arf1, ArfGAP1 and an Arf1 exchange factor, this combination of components gave rise to a self-organized membrane system in which the giant liposomes were encircled by small ones.

The work of Drin et al. is a beautiful illustration of a self-organization module that ensures the oriented tethering of membranes until the properties of these membranes change. This concept provides an ideal solution to the problem of how the Golgi complex maintains its structure in the face of a massive flow of membrane through the organelle. Here it is the shape of the membrane structures themselves that dictate the subsequent step in a trafficking pathway – fusion of the two membranes. How this elegant mechanism is integrated into the flow of traffic, along with its morphological transitions, at the ER–cis-Golgi interface is an important issue that now needs to be answered. GMAP-210 has been shown to localize to and function in trafficking between the ER and cis-Golgi. It is now pertinent to investigate what vesicles the N-terminal ALPS motif binds to, with likely candidates being COPII- or COPI-coated vesicles. Another issue is whether the ALPS motif binds through the coat to the lipid surface or only binds after vesicle uncoating. One plausible model is that GMAP-210 helps to assure correct delivery of vesicles to a specific region of an acceptor-compartment membrane on which Arf1 has been activated (Fig. 3C). Reduction of GMAP-210 levels in mammalian cells by RNA interference causes fragmentation of the Golgi ribbon, indicating an important role for GMAP-210 in maintaining Golgi structure in vivo (Rios et al., 2004). However, depletion of GMAP-210 in *Drosophila melanogaster* had only mild effects on Golgi structure, and hence the in vivo roles of GMAP-210 remain to be established (Friggi-Grelin et al., 2006). A complete response to these questions will ultimately depend on determining the precise molecular events that occur in trafficking at the cis-Golgi, an issue that is still far from resolved. However, a framework for understanding trafficking through the secretory pathway is emerging, with a significant advance contributed recently through the development of a new model for intra-Golgi trafficking.

Mechanisms of trafficking through the Golgi

The anterograde vesicular trafficking model was the favored paradigm for trafficking through the secretory pathway for a number of years (Palade, 1975). In this model, it was postulated that coatomer-coated vesicles carried cargo forwards from one compartment to the next (Fig. 4A). This simple and straightforward model has received considerable support for the first trafficking step of the secretory pathway, from the ER to the first post-ER compartment, which is mediated by the COPII coat (Lee et al., 2004). However, when the predictions of this model were tested for COPI-dependent intra-Golgi trafficking steps, it became clear that it did not adequately account for the experimental observations. For example, purified intra-Golgi COPI vesicles were found in some cases to be enriched in glycosylation enzymes, not cargo (Martinez-Menarguez et al., 2001; Rabouille and Klumperman, 2005).

The cisternal-maturation model provided a much better fit to the experimental data, and represented a return to the preferred model that was derived from early morphological studies (Morré and Mollenhauer, 2007) (Fig. 4B). Indeed, for large cargo aggregates, such as collagen, a number of EM studies seemed to provide evidence for these large aggregates remaining in cisternae as they traversed the Golgi stack (Bonfanti et al., 1998; Clermont et al., 1993; Melkonian et al., 1991; Mironov et al., 2001). Proof for the idea of
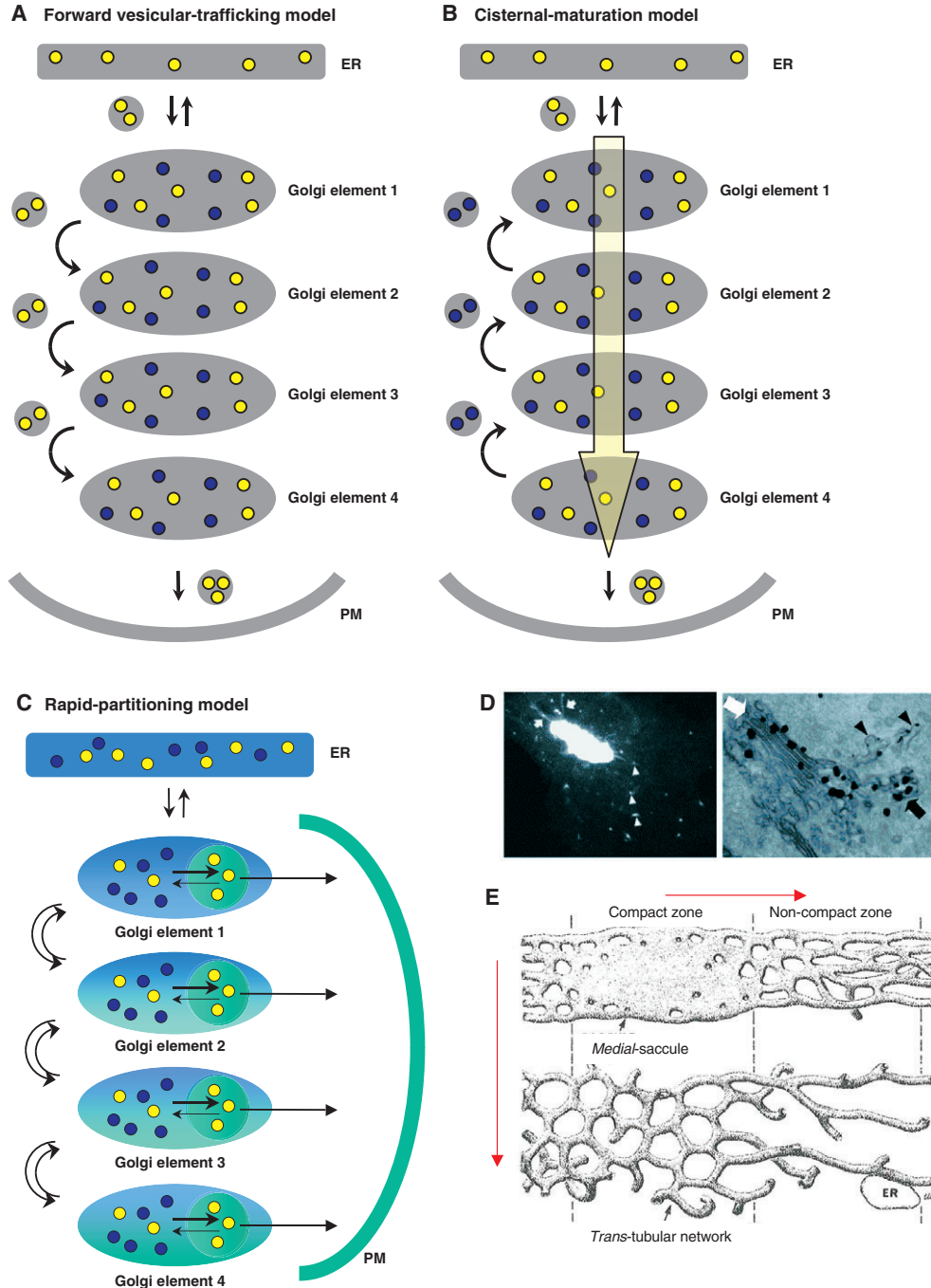
Fig. 4. Models for intra-Golgi trafficking. Cargo synthesized in the ER and transported through the secretory pathway is shown in yellow; Golgi processing enzymes are shown in blue. Golgi element 1 is on the *cis* side and receives material from the ER, and Golgi element 4 is on the *trans* side and is involved in packaging of cargo for delivery to the plasma membrane (PM). Arrows indicate the direction of trafficking. (A) Forward vesicular-trafficking model. Vesicles carrying cargo bud from a donor compartment, and are then targeted to and fuse with the following compartment in the secretory pathway (acceptor compartment). (B) Cisternal-maturation model. Vesicles carrying Golgi processing enzymes bud from a later compartment in the secretory pathway (donor compartment), then fuse with an earlier compartment (acceptor compartment). The cargo progresses as a result of maturation of an earlier compartment into a later one. (C) Rapid-partitioning model. Cargo exits the ER and is transported to the *cis*-Golgi. Once in the Golgi, cargo can move in a bidirectional manner, both in the *cis*-to-*trans* and *trans*-to-*cis* directions. Golgi glycosylation enzymes are synthesized in the ER, then move to the Golgi via forward vesicular trafficking where, similar to the cargo, they can then move in either direction up and down the Golgi stack. Blue, GPL-enriched membranes; green, SL-enriched membranes. Green circles within each Golgi element represent export domains that are enriched in SL. (D) Correlative fluorescence immuno-EM showing Golgi-PM carriers arising from tubular regions of the Golgi. Upper panel: fluorescence microscopy image of a VSVG-GFP-transfected cell. Golgi-PM precursors (arrows) and mature Golgi-PM carriers (arrowheads) are shown. Lower panel: immuno-EM image of the same cell shown in the upper panel, labeled for VSVG by the enhanced immuno-gold method. A Golgi-PM precursor structure still attached to the *trans*-Golgi (black arrow) and mature Golgi-PM carriers (arrowheads) arise from a tubular region of the Golgi. White arrow points to the saccular compact region of the Golgi. Reproduced with permission from Luini et al. (Luini et al., 2005). (E) Drawing of a *medial*-Golgi saccule (upper panel) and a *trans*-Golgi tubular element (lower panel). The compact and non-compact zones are indicated. Red arrows indicate the direction of transitions from saccular to tubular, which occurs in both the *cis*-to-*trans* direction across the Golgi stack (vertical arrow) and from the compact to non-compact zones at a single level of the Golgi (horizontal arrow). Reproduced with permission from Rambourg et al. (Rambourg et al., 1979).

membrane maturation within the Golgi, in the sense of one set of Golgi-resident proteins replacing another, came with two landmark papers using live-cell imaging of yeast Golgi (Losev et al., 2006; Matsuura-Tokita et al., 2006). In these papers, the replacement of an early Golgi marker by a later Golgi marker on the same membrane structure in real time was visualized directly (Fig. 2C). The dispersed organization of the Golgi in yeast allowed visualization of these maturation events, which would not have been feasible in higher eukaryotes. In mammalian cells, Golgi precursors travel along microtubules towards a centrosomally localized single Golgi structure, where they fuse with *cis* cisternae to a form continuous, steady-state system with constant input and output. In yeast, trafficking through the Golgi is microtubule independent, and Golgi elements arise next to ER exit sites, which are scattered throughout the cell; here, the Golgi elements mature and then dissipate without converging into a larger, steady-state system. In addition, the elements of the yeast Golgi do not contain multiple compartments from *cis* to *trans*, but are quite simple and often contain only one or two different compartment markers at a given time. The exploitation of these unique properties of the yeast Golgi structure allowed the Glick and Nakano laboratories to unequivocally prove that a single Golgi membrane element undergoes a transition from a *cis* composition to a *trans* composition over time.

The motivation for the development of a new model for trafficking through the Golgi came from studies of the dynamics of cargo transport in living cells (Patterson et al., 2008). The classic cisternal-maturation model predicts that cargo entering the Golgi will remain there for a fixed amount of time while glycosylation enzymes are delivered sequentially via retrograde trafficking to the compartment in which the cargo molecules are located (Fig. 4B). In this model, a lag period after entry of the cargo into the Golgi is predicted. Using five different fluorescent-protein-tagged cargo molecules, Patterson et al. examined the kinetics of cargo exit, and found that, in contrast to the predictions of the cisternal-maturation model, all cargo exhibited exponential exit kinetics from the Golgi with no initial lag period upon entering the Golgi (Patterson et al., 2008). These data could not be reconciled with the cisternal-maturation model either in its classic form or in any modification of the model, including very rapid Golgi-resident-enzyme recycling. Using the temperature-sensitive VSVG protein fused to GFP (GFP-VSV-ts045G; hereafter referred to as VSVG), Patterson et al. verified by EM analysis that cargo molecules were distributed throughout the Golgi within 5 minutes of release from the ER, and were present in both the saccular compact regions and the vesiculotubular non-compact regions of the Golgi, as has been observed previously (Martinez-Menarguez et al., 2001; Mironov et al., 2001; Patterson et al., 2008).

Using a pulse-chase regime, Patterson et al. found that, upon arrival at the Golgi, VSVG rapidly distributed into domains of the Golgi that contained the processing enzyme galactosyltransferase (GalT), but then concentrated in regions that were spatially distinct from the GalT-enriched domains. Transport carriers that were destined for the PM emerged from the VSVG-enriched domains, consistent with their being specialized domains for export from the Golgi. Further proof of this idea came from cells treated with BFA. Interestingly, only a portion of VSVG was redistributed into the ER upon BFA treatment, with a significant amount remaining in the Golgi region. Packaging of this pool of VSVG into transport carriers that trafficked to the PM continued in the presence of BFA. This BFA-resistant portion of VSVG did not colocalize with a *trans*-Golgi network (TGN) marker, TGN38, indicating that it was not

present in the TGN. This result is consistent with previous observations that the TGN, which is specialized for transport to the endosomal-lysosomal system, is distinct from regions of the *trans*-Golgi that give rise to Golgi-PM transport intermediates (Ladinsky et al., 1999; Mogelsvang et al., 2004).

The rapid-partitioning model

The model proposed by the Lippincott-Schwartz laboratory – the rapid-partitioning model – to explain their novel observations has three key elements: sorting of lipids into different domains at each level of the Golgi; bidirectional trafficking of cargo and processing enzymes within the Golgi; and association of proteins with their preferred lipid environment (Fig. 4C). In addition to the standard trafficking pathways between the ER and the Golgi both for cargo and for glycosylation enzymes, the model postulates trafficking of both resident Golgi enzymes and cargo in both directions through the Golgi (Fig. 4C). This bidirectional trafficking is a departure from the existing cisternal-maturation model, which assumes active trafficking of only the glycosylation enzymes and only in the *trans*-to-*cis* direction (Fig. 4B). However, bidirectional trafficking within the Golgi has been proposed previously (Pelham and Rothman, 2000).

A major novelty of the rapid-partitioning model is the inclusion of lipid-trafficking pathways as an integral part of the model. The two major classes of lipids within the Golgi are glycerophospholipids (GPLs) and sphingolipids (SLs), and their trafficking routes differ significantly. GPL synthesis takes place in the ER and forward secretory transport carries these lipids to the Golgi. GPL transport is expressed as the sum of constitutive and cargo-load-dependent components, to account for the fact that more lipid is required to package a higher cargo load for transport to the Golgi (Patterson et al., 2008). GPLs also traffic back from the Golgi to the ER through retrograde membrane-trafficking routes that recycle trafficking machinery and glycosylation enzymes (Bonifacino and Glick, 2004; Rabouille and Klumperman, 2005). SLs, unlike GPLs, are synthesized in the Golgi (van Meer et al., 2008). Although the precursor for their synthesis, ceramide, is produced in the ER, it is not transported to the Golgi along with cargo in a vesicular pathway, but is instead transferred directly to the *trans*-Golgi by a lipid carrier protein, CERT (D'Angelo et al., 2007; Halter et al., 2007). The synthesis of SLs exclusively in the Golgi is a key parameter of the rapid-partitioning model – in simulations in which SL was delivered along with GPL and cargo from the ER to the *cis*-Golgi, known features of the Golgi were not produced (Patterson et al., 2008). It is noteworthy that the synthesis of SLs uniquely in the Golgi is conserved from yeast to mammals (Levine et al., 2000).

The second important aspect of the rapid-partitioning model is the existence of two types of domain at each level of the Golgi: one enriched in GPLs and Golgi processing enzymes, and one enriched in SLs that has a higher concentration of cargo. It is postulated that cargo enters carriers that are targeted to the PM uniquely from the SL-enriched domains. This idea is consistent with observations that SLs are delivered to the PM by transport carriers that are formed at the Golgi (Helms and Zurzolo, 2004; Rodriguez-Boulan et al., 2005; van Meer et al., 2008). SL-enriched domains can be found at all levels of the Golgi, including early-Golgi compartments (Helms and Zurzolo, 2004). Morphological studies have shown that cargo can exit the Golgi from the *cis* side (Mogelsvang et al., 2004; Volchuk et al., 2000), and the initial stages of the formation of secretory granules can take place in the

cis-Golgi (Kepes et al., 2005). A reasonable hypothesis for the location of the SL-enriched export zones in the mammalian Golgi is in the non-compact tubular regions that connect the saccular compact zones, and also in the tubular *trans*-Golgi regions (Fig. 4D,E). EM analysis supports this hypothesis by demonstrating that Golgi-PM carriers arise from tubular regions of the Golgi (Polishchuk et al., 2003) (Fig. 4D). Each saccule of a compact zone is linked to the tubulovesicular non-compact zone (Fig. 1, Fig. 4E) and, hence, the existence of export domains within the tubulovesicular non-compact regions of the Golgi would satisfy the requirements of the rapid-partitioning model through their connection with every Golgi cisterna. The non-compact regions of the Golgi contain both COPI and clathrin-coated structures, and are thus involved in both early- and late-Golgi sorting processes (Ladinsky et al., 1999; Mogelsvang et al., 2004). Furthermore, Mironov et al. have demonstrated an important role for the non-compact tubular regions in secretion by using the drug dicumarol, which blocks secretion within the Golgi and causes the tubulovesicular non-compact regions of the Golgi to break down, leaving the stacks intact but no longer connected (Mironov et al., 2004).

The final tenet of the model is that both cargo and glycosylation enzymes have an optimal lipid environment with which they preferentially associate within the Golgi. Rapid bidirectional trafficking throughout the Golgi system allows proteins to sample different environments, and therefore promotes the association of each protein with its optimal Golgi subdomain. This idea has been proposed previously for the localization of glycosylation enzymes within the Golgi by the Munro laboratory, based on observations that the length of the transmembrane (TM) domain of a single-span TM protein has a major role in its localization [see Munro and references therein (Munro, 1995)]. Glycosylation enzymes that reside in the Golgi have TM domains that are on average five amino acids shorter than PM-resident proteins, which correlates with the biophysical properties of their resident membranes (Lundbaek et al., 2003). As proposed by Munro and Bretscher, 'the notion of protein sorting by lateral partitioning between coexisting lipid domains has the particular appeal that it is inherently self-organizing, thereby escaping the requirement for further proteins that would themselves have to be retained in place' (Bretscher and Munro, 1993). Subsequent work has lent strong support to the idea that TM-domain sorting occurs throughout the secretory pathway (de Planque and Killian, 2003; Lundbaek et al., 2003; Ronchi et al., 2008).

Although lipid-based sorting is a major determinant in the localization of glycosylation enzymes within the Golgi, protein-mediated mechanisms also exist. Indeed, Golgi localization of glycosylation enzymes requires not only the TM domains but also determinants within both the luminal and cytoplasmic regions (Opat et al., 2001). Recent work has shed light on the mechanism by which the cytoplasmic portions of Golgi-resident glycosyltransferases mediate Golgi localization. Vps74p binds directly to the cytoplasmically oriented C-terminal tails of numerous glycosylation enzymes in yeast, and is responsible for retaining and organizing these enzymes within the Golgi through an additional interaction with the COPI coat (Schmitz et al., 2008; Tu et al., 2008). Vps74p has mammalian homologs, the GmX33 (Gpp34) proteins, and hence the Vps74p-mediated mechanism for the retention of enzymes in the Golgi is evolutionarily conserved to some extent, although protists and plants appear not to have an obvious ortholog (Tu et al., 2008).

The rapid-partitioning model invokes gradients of lipid composition in two directions within the Golgi: in the *cis*-to-*trans* direction across the Golgi, and also within each cisterna. Strikingly,

there is a morphological correlate to the formation of lipid gradients along these two axes of the Golgi. First, from the *cis*- to the *trans*-Golgi, there is a progression from mostly saccular membranes to the tubular networks that are characteristic of the *trans*-Golgi and TGN (Fig. 4E). Second, at a given level of the Golgi, there is a transition from saccular to tubular membranes, going from a compact to a non-compact zone (Fig. 4E). It is therefore an interesting possibility that the formation of tubulovesicular regions of the Golgi, which are conserved from yeast to mammals, is required for lipid sorting and that ER export domains arise within these tubulovesicular regions.

Mechanisms of lipid sorting

The key process in the rapid-partitioning model is lipid sorting. Patterson et al. propose that the formation of SL-enriched domains could occur by phase separation at a crucial concentration of SL to GPL within Golgi membranes. Artificial lipid bilayers that are made up of GPLs that are similar to those in biological membranes are in a liquid disordered (L_d) phase, whereas those that contain both SLs and cholesterol can form a distinct phase that is known as the liquid ordered (L_o) phase; the saturated acyl chains of the SLs in the L_o phase are tightly packed together with intercalated cholesterol, forming an ordered structure with high lateral mobility (Feigenson, 2006; Jacobson et al., 2007; van Meer et al., 2008). In model artificial membranes that contain GPL, SL and cholesterol, when the mole percent of SL with respect to GPL is increased at a given concentration of cholesterol, phase separation occurs at a specific SL:GPL ratio (Feigenson, 2006). The range of concentrations at which SLs and cholesterol undergo phase separation in model systems corresponds to their concentrations in Golgi membranes, so it is theoretically possible that this type of phase separation occurs in cells (Jacobson et al., 2007). Although advances are being made in the detection of membrane domains within the PM, the existence of L_o domains is more controversial and, in any case, L_o domains have never been observed within Golgi membranes (Feigenson, 2006; Jacobson et al., 2007; Munro, 2003). Hence, experimental proof of the idea that lipid-phase transition is the mechanism that leads to the formation of SL-enriched export domains within the Golgi will have to await the development of new technology.

Lipid-phase separation is not the only mechanism by which lipid sorting could take place, however. Tubulovesicular COPI structures have a low SL content compared with the Golgi membranes from which they are derived (Brugger et al., 2000). In an in vitro reconstituted system using giant unilaminar vesicles (GUVs) that contain SLs and GPLs, it has been shown recently that COPI protects regions of these GUVs from undergoing phase separation, providing further proof of a role for COPI in lipid sorting (Manneville et al., 2008). Therefore, another possibility is that the sorting of SLs into export domains occurs through a coatomer-mediated process. Another potential mechanism is membrane-curvature-mediated lipid sorting. Highly curved membrane tubules that are formed from GUVs in vitro exclude SLs and are enriched in GPLs (Roux et al., 2005). These results are consistent with the idea that SL molecules, with their long, saturated acyl chains, do not easily enter the regions of high curvature that are induced by the formation of a vesicle or tubule (Antonny, 2006). This property of SLs could explain why Golgi-PM carriers are large containers and not small vesicles. Hence, curvature-mediated lipid sorting would provide an ideal mechanism for the formation of Golgi-PM carriers within tubular networks. How membrane curvature is generated is the subject of intense interest, and multiple mechanisms have been identified. Amino-phospholipid translocases (flippases) that alter the phospholipid ratio

between the two leaflets of the bilayer can directly generate membrane curvature (Graham, 2004). The insertion of amphipathic helices, particularly those that have bulky hydrophobic residues, can cause the membrane to bend, as has been demonstrated for the N-terminal helix of the yeast protein Sar1p (Antonny, 2006; Lee et al., 2005). The banana-shaped BAR domain provides another example of a curvature-inducing motif, which bends the membrane to conform to its concave shape, in some cases aided by an associated amphipathic helix. Finally, membrane deformation into highly curved structures by the COPI coat is regulated by membrane tension (Manneville et al., 2008). In this study, the authors showed that GUVs under high tension could bind Arf1-GTP and COPI, but could not form COPI budding profiles. Hence, COPI and Arf1-GTP alone are not sufficient to form COPI buds or vesicles, but the lipid membrane must be in the correct physical state to allow formation of these highly curved structures (Manneville et al., 2008).

Conclusions and perspectives

The rapid-partitioning model provides a new framework for understanding transport through the Golgi complex, and emphasizes the important role of lipids in driving trafficking through this organelle. The model postulates that the Golgi forms a morphologically continuous system, but does not invoke a single well-mixed compartment. On the contrary, spatially distinct compartments of the Golgi are an essential aspect of the model, with rapid trafficking of material between these laterally segregated membrane domains. Regulated fission and fusion events, which are essential for trafficking through the Golgi in all systems from yeast to mammals, are likely to be important for maintaining segregated lipid domains within an anatomically continuous Golgi structure. These events require the formation of highly curved membrane structures, and the fact that there are protein modules such as the BAR domain and the ALPS motif – that detect membrane curvature directly – reveals the importance of membrane-curvature changes in membrane-trafficking pathways throughout the cell (Antonny, 2006; McMahon and Gallop, 2005). The existence of ALPS motifs in a wide range of proteins that regulate trafficking in the ER-Golgi system (Drin et al., 2007) indicates that curvature sensing is an important general feature of trafficking in the secretory pathway.

The idea of membrane maturation, which has been demonstrated directly in yeast, is not at all excluded by the rapid-partitioning model. On the contrary, rapid changes in the lipid and protein content of a given membrane compartment over time would be consistent with the sorting processes that are an integral part of the model. The rapid-partitioning model predicts that spatial segregation of SL and GPL synthesis, in the Golgi and ER, respectively, is essential for establishing a lipid gradient across the Golgi. This idea is attractive in that it provides an explanation for the existence of numerous ER-Golgi transfer proteins, such as CERT and FAPP2, that directly transfer precursors of SLs from the ER to the Golgi (De Matteis and Luini, 2008). SL synthesis within the Golgi is conserved in yeast, and in this genetically tractable organism it should be possible to test whether Golgi localization of SL synthesis is important for trafficking through the Golgi. The fact that Golgi-membrane maturation can be visualized directly in this organism provides a powerful tool for testing this and other predictions of the rapid-portioning model.

The model does not explicitly include the trafficking of cholesterol, and this will be an important molecule to consider in the future. Cholesterol is found in both GPL- and SL-enriched regions of the Golgi, although it concentrates in SL-enriched membranes,

and both ER-Golgi and intra-Golgi trafficking require the appropriate concentration of membrane cholesterol (Helms and Zurzolo, 2004; Ridsdale et al., 2006; Stuven et al., 2003). Cholesterol has a profound effect on the properties of membranes, and is likely to be important for providing the correct environment for the accommodation of different types of TM proteins within the membrane (Bretscher and Munro, 1993; Lundbaek et al., 2003).

Numerous studies have indicated the important role of lipids such as phosphoinositides in providing identity to different intracellular membrane compartments and in signaling processes (De Matteis and Luini, 2008). We have, for the first time, a demonstration of how membrane shape is used by the cell to regulate membrane association prior to fusion (Drin et al., 2008). That protein trafficking itself might be driven by properties of membrane lipids, as predicted in the rapid-partitioning model (Patterson et al., 2008), emphasizes to an even greater extent the importance of studying the interplay between proteins and lipids in elucidating the mechanisms of intra-Golgi trafficking.

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