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Structural aspects of Golgi function

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Abstract. Since its discovery, the Golgi complex has attracted the attention of cell biologists because of its 'fashionable' morphology and central position within the secretory system of the cell. Here, we discuss how the three-dimensional architecture of the Golgi complex relates to its multiple functions in protein sorting and processing, and how an analysis of the morphology of the Golgi complex can help to provide an understanding of the mechanisms involved in transport through this unique organelle.

Key words. Golgi complex; intracellular transport; morphology.

Introduction

The Golgi is the central station of the secretory pathway

All cells secrete a variety of macromolecules in order to modify their environment or to protect themselves. At the same time, cells synthesize new proteins and lipids to replace those that are consumed within the various compartments of the biosecretory and endocytic pathways. During their synthesis, proteins are transferred from ribosomes into the endoplasmic reticulum (ER) lumen or incorporated into the ER membrane, and (after their folding) transported along the biosecretory pathway. Then, newly synthesized proteins and lipids are transported to the Golgi complex, from where they move to their final destinations [1].

Over the last 20 years or so, the field of intracellular membrane transport has seen tremendous advances towards the identification of the relevant molecular machineries and their deciphering at the level of their molecular interactions, often by using completely artificial and well-controlled membrane systems [2–4]. In contrast, surprisingly, a satisfactory understanding of how transport occurs in vivo at the organelle level has not been achieved. Similarly, many mechanistic questions relating to the morphology of the Golgi remain open. As a consequence, our present view of the overall mode of transport through the Golgi and the related mechanisms of the regulation of Golgi structure are poorly developed. The reasons for this lag are both technological and conceptual. Technological, because intra-Golgi traffic is an extremely dynamic event which occurs within a very limited space. The main problem in understanding Golgi morphology is the limitation of the spatial resolution of methods used for the analysis of Golgi structure. On the one hand, biochemical isolation allows dissection of different compartments of the Golgi, but it also leads to loss of integrity and its ribbonlike morphology. On the other hand, the more recent and popular fluorescent protein (FP) video imaging has been hampered by the resolution of light microscopy. EM, unfortunately, is not able to provide similar time-lapse analyses of living cells. However, the combination of recent techniques, such as FP-based analysis in living cells, fluorescence resonance energy transfer (FRET), correlative light-electron microscopy (CLEM) and EM tomography (EMT), has provided new insights into the morpho-functional organization of the Golgi.

From a conceptual point of view, the field has been dominated until very recently by the anterograde vesicle model of intra-Golgi traffic [5]. This restricted the research efforts of many laboratories to mechanistic analyses of the protein machineries residing in the Golgi complex. An understanding of how these complex machineries are orchestrated within such a complicated membrane structure as the Golgi has been absent. This

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has led to some confusion and, as a result, to the existence of several models of intra-Golgi transport. Parts of these possible models are suitable for an analysis at the current level of knowledge, while others remain too problematic [6]. Indeed, it has now become clear that the vesicular model is not correct [7], as will be discussed further below.

Strikingly, even 105 years after the original Camillo Golgi discovery, we know very little about this organelle. As we have substantial experience in the structural analysis of intra-Golgi transport, we herein review the current knowledge of the morphology of the Golgi with respect to the various transport models. In doing so, we would like to stress that this review is focused on the functional aspects of Golgi morphology mostly with regard to mammalian cells. Therefore, we apologize from the start to those authors whose work may not be cited here due to space limitations.

A functional view of the Golgi

The Golgi is the central station along the secretory pathway. It receives newly synthesized proteins and lipids from the ER, and then distributes them to the plasma membrane (PM) and to the endosomal/lysosomal system [8]. In polarized cells (e.g. epithelial cells and neurons), it sends proteins and lipids to the correct surface domains [8]. The Golgi also serves as a 'factory' for the posttranslational modification of proteins and lipids (mostly by glycosylation). In the lumen of the Golgi, glycosyltransferases bind to the cargoes and transfer new monosaccharides to their oligosaccharide chains. Glycosyltransferases act sequentially, such that the oligosaccharide chain itself serves as a preferred acceptor substrate for the subsequent glycosyltransferase. In general, enzymes acting early in glycan biosynthetic pathways have been seen to be localized to *cis* and medial compartments of the Golgi [9], whereas enzymes acting later in the biosynthetic pathway tend to reside within the *trans*-Golgi cisternae and the *trans*-Golgi network (TGN; see below and fig. 1) [10].

Many mammalian glycosyltransferases are themselves posttranslationally modified by glycosylation. The first enzyme beginning this process of glycosylation after the ER is mannosidase I (Man I) [11]. Thus, the Golgi begins from the membranous domain enriched in Man I and finishes in the domain enriched in sialyltransferase, fucosyltransferase or sulphatases [10]. Why and how cells isolate Golgi glycosyltransferases from other transferases of the ER is not clear. Apart from the glycosyltransferases, the Golgi contains numerous pro-protein convertases (like furin) that cleave protein precursors into their mature forms [12].

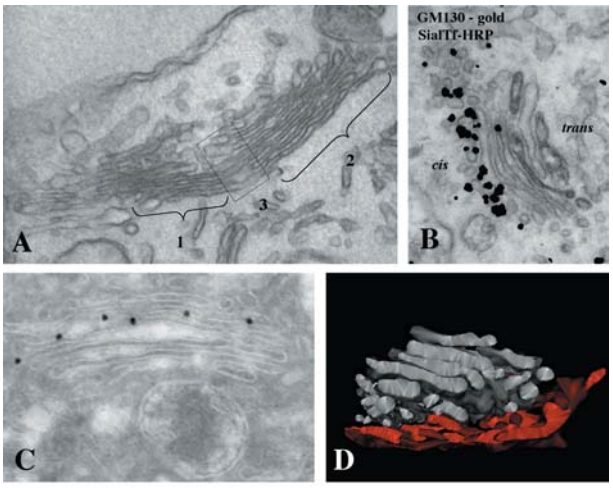

Figure 1. Ultrastructure of the Golgi complex. (*A*) Thin section of NRK cells showing a fragment of the Golgi ribbon which comprises two stacks (1, 2) connected through a noncompact zone (3). (*B*) Polarity of the Golgi stack is elucidated by immunogold staining of the *cis*-marker GM130 and HRP-based detection of the *trans*-Golgi enzyme, sialyltransferase. (*C*) Immunogold detection of galactosyltransferase reveals the distribution of the enzyme through the *trans* cisternae of the Golgi stack. (*D*) Three-dimensional view of a Golgi stack reconstructed from the thin serial sections.

The structure of the Golgi

Position within the cell

In mammalian cells, the Golgi is typically located around the centrosome, where it remains due to a microtubule (MT)-dependent mechanism. Indeed, the Golgi undergoes fragmentation into hundreds of 'islands' (known as mini-stacks) when MTs are depolymerized by specific drugs (e.g. nocodazole) [13]. Upon nocodazole washout, the Golgi fragments immediately undergo centralization, and the ribbon of interconnected stacks is rebuilt [14]. Polarization of the cell leads to the reorientation of the centrosome and a relocation of the Golgi. For example, both the Golgi and the centrosome are oriented towards the leading edge of migrating fibroblasts [15] and towards the immune synapse in cytotoxic T lymphocytes when they kill their targets [16]. In polarized epithelial cells, the centrosome is positioned near the apical portion of the cell surface [17], where the Golgi also resides. A special isoform of kinesin (KIFC3) has been shown to play a role in this MT-dependent Golgi centralization and positioning [18]. Of interest, most of the Golgi elements are situated at a distance of 1–3 μm from the centrosome (fig. 1; [our unpublished observations]). The reason for this is not clear, although it is probable that the MTs growing from the centrosome at a high-density 'push' the Golgi membranes out, forcing them to stay at some distance from the cell center.

General structure

Schematically, this organelle consists of flat cisternae grouped into several stacks (compact zones) that are interconnected by tubular networks (noncompact zones), which together form a continuous membranous ribbon (fig. 1A) collected in a pericentriolar space [19]. For a long time the isolated stacks were viewed as an essential component of the Golgi (see Farquhar and Palade [20]). More recently, however, a variety of approaches, including high-resolution scanning EM [21, 22], stereopair analyses of thick sections [19, 23] and computer-aided reconstructions of serial thin sections [24], have allowed different groups to clearly demonstrate that the tubular-reticular areas also play an essential structural role in the Golgi organization, connecting neighboring stacks of cisternae to each other (fig. 1). Recent EMT analyses have further confirmed this Golgi organization, and no completely isolated cisterna has been seen [25, 26]. The interconnections between the Golgi stacks have been confirmed by functional experiments in living cells, where laser bleaching of parts of the Golgi containing fluorescently tagged Golgi resident proteins quickly induces the loss of fluorescence from other parts of the Golgi, through diffusion between nonbleached and bleached zones [27]. Despite this architecture, the Golgi apparatus is highly dynamic and can combine enormous rates of membrane flux with the ability to rapidly change its shape, and to even disassemble and reassemble, under a variety of physiological and pathological conditions [5]. Apparently this plasticity of the Golgi membranes relies on the numerous molecular machineries present in this organelle. However, we will address the role of these molecular machineries only partially; the central questions of the present report/discussion are how such a complex architecture of the Golgi is related to its transport function.

The compartments of the Golgi

The Golgi can be schematically viewed as being composed of three main compartments: the *cis*-, medial and *trans*-Golgi [28] (fig. 1B, C), with three basic structural elements: stacks of flat disc-shaped cisternae, abundant tubular-reticular networks and vesicles [19, 29]. At the *cis* side of the Golgi stacks there is a tubular network that is known as the *cis*-Golgi network (CGN), which is composed of branching tubules connected with the *cis*-most cisterna, which itself is usually highly perforated. In neurons, the *cis*-most cisternae form a continuous layer over several stacks [19]. The CGN is followed by the stack of flat cisternae (fig. 1D). Finally, at the *trans* side of the stacks there is another structure that often appears as a network of branching tubules on EM sections, the *trans*-Golgi network (TGN). The lipid content of these different Golgi compartments also differs significantly [30–32], with most of the cholesterol being concentrated at the *trans* side.

The *cis*-Golgi

Three dimensional (3D)-tomography reconstruction has revealed that the space near the *cis* side of the Golgi stacks contains a layer of branching tubular structures and flattened saccules (potentially ER-to-Golgi carriers [EGCs]). These tubular-saccular organelles exhibit membrane domains resembling budding or fusing vesicles, none of which appear to be coated [25]. The origin of free COPI vesicles found at the *cis* face of the Golgi is not clear. Potentially such vesicles can form from the elements of the CGN; however, it is more likely that they belong to transport carriers arriving from the ER. Tubular elements of the CGN are often seen to be continuous with the *cis*-most flattened elements of the cisternae of the compact region of this organelle. Why the CGN is tubulated also remains to be understood. One possibility is that the tubules emerging from the *cis*-Golgi participate in the recycling and transport of different molecules back to the ER. Live-cell imaging clearly suggests that KDELR, ERGIC53, Golgi enzymes [33], Shiga toxin and Rab6 [34] can all be observed within the tubules moving from the Golgi back to the ER. The other possibility is that the membranes of the *cis*-Golgi receive carriers arriving from the ER that comprise a significant tubular component.

There are several possible mechanisms for the entrance of cargo into the Golgi (fig. 2). The oldest scenario suggests that secretory proteins arrive at the *cis*-Golgi within vesicles from the intermediate compartment (IC) (fig. 2A), which in turn receives vesicles budding from the ER [35]. Now it is clear, however, that the IC is not a static compartment positioned somewhere at the ER-to-Golgi interface. It apparently comprises structurally complex EGCs that emerge from the ER and move towards the Golgi along MTs. Another mechanism implies direct fusion of these EGCs with *cis*-Golgi membranes (fig. 2B) [6, 36], although there is also the possibility that EGCs do not fuse with the *cis*-Golgi, but fuse with each other instead (fig. 2C), thus forming a new *cis*-Golgi cisterna which can then progress through the Golgi stack [37]. Finally, we cannot exclude the possibility that the membrane tubules that usually contain GM130 and can grow from the Golgi sometimes for dozens of microns [38] are able to arrive directly at the ER exit sites (fig. 2D), fuse with forming EGCs, and then 'pump' their contents into the Golgi via a 'bolus-like' mechanism [39].

The Golgi relationship with the ER

Membranes of the Golgi complex and the ER are usually intimately associated. Over many years, EM studies have revealed that the ER is frequently positioned close to the *trans*-most cisternae or the tubules of the TGN [25, 26, 29, 40]. Moreover, smooth cisterna of the ER (without ribosomes) have sometimes been seen to be partially in-

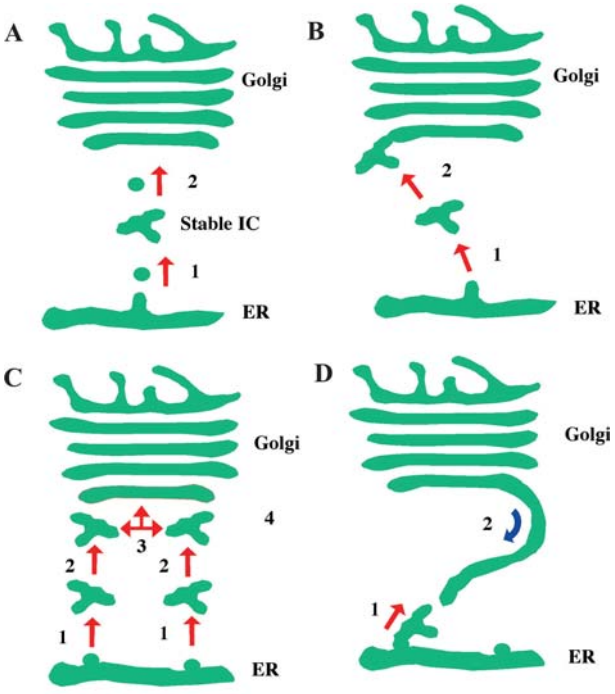

Figure 2. Possible mechanisms of cargo arrival at the Golgi complex. (*A*) Newly synthetized cargo proteins are transported by vesicles from the ER to the stable IC (1), and then another population of vesicles carries the cargo from the IC to the Golgi (2). (*B*) Transport carriers emerge at the ER exit sites (1) and then move to the Golgi (2), where they fuse with the cisterna of the stack. (*C*) Transport carriers emerge at the ER exit sites (1) and then move to the Golgi (2), where they fuse with each other (3) to form a new cisterna of the stack (outlined in red). (*D*) Membranes budding at the ER exit sites (1) fuse with a tubular outgrowth (2) of the Golgi membranes in order to transfer cargo through the direct ER-to-Golgi connection.

serted between the two *trans*-most cisternae of Golgi stacks. Such a close association of the *trans*-Golgi and the ER has been proposed to play a role in lipid and ion exchange between the two organelles [25], although its real purpose still remains unclear. At the other pole of the Golgi stack, direct membrane connections between the Golgi cisternae and the ER have frequently been found, even in single random thin sections (for a review, see Mironov et al. [41] and below). Apparently, the Golgi and the ER might also be connected via elements of the IC. Indeed, IC tubules are occasionally seen to be in continuity with the ER, while their connection with the CGN has been well documented [29].

The question of the functional role of these connections has still to be answered. ER-to-Golgi connections could be involved both in anterograde transfer of cargo from the ER and retrograde Golgi-to-ER transport. Unfortunately, EM data do not provide sufficient information regarding the molecular composition of such connections. The Sar1p GTPase (a component of the COPII machinery) has been shown by immuno-EM (IEM) to be on tubules connecting the ER and EGCs [42, 43]. However, it is not clear whether and how Sar1p tubules reach the Golgi. On the other hand, GM130 tubules visualized in living cells have been shown to participate in anterograde transport of cargo [38], although the issue of whether these tubules connect the Golgi and the ER still remains to be addressed. With respect to retrograde transport, Golgi-derived tubular structures have been shown to be involved in this process (see above). Indeed, several lines of evidence suggest that Golgi enzymes might use membrane connections for Golgi-to-ER recycling. For example, in cells with depolymerized MTs, the Golgi islands undergo reassembly at the ER exit sites [44, 45], and therefore such rearrangements increase the chance that Golgi stacks establish connections with the ER [our unpublished ETM observations]. Under these conditions, Golgi enzymes fused with green fluorescent protein (GFP) have been seen to move from the Golgi to the ER at a relatively fast rate, indicating that there is recycling of Golgi enzymes to the ER [46]. With the consideration that COPI vesicles, which are thought to serve as retrograde transport carriers, do not contain Golgi enzymes (see below), the obvious proposal is that Golgi enzymes move to the ER by lateral diffusion along ER-to-Golgi membrane continuities or by retrograde tubules, as has been suggested by Sciaki et al. [33].

The medial Golgi (the central Golgi domain)

In our view, the medial Golgi comprises all cisternae of the stack located between the CGN and the TGN, and that has been referred to as the 'central Golgi domain' [47]. The *cis*-most and *trans*-most cisternae of the Golgi stack can therefore be considered as the CGN-to-stack and the stack-to-TGN transitional elements, respectively. Apart from the cisternae, the medial Golgi possesses significant tubular and vesicular structural elements.

The stacks of cisternae

The number of cisternae forming the Golgi stacks varies from one cell type to another (three to eight cisternae, in the majority of cases), while the length of the cisternae frequently depends on the axis of the section. The lumen of a cisterna is usually quite narrow (10–20 nm) but may be uniformly dilated. Cisternae may show swellings filled with a material of low electron density, known as pro-secretory granules. However, cisternae usually appear as very flattened and wide tubules, or as irregular parallelepipeds with semicylindrical rims. Such a geometry might allow an enhancement of the efficiency of glycosylation [28], considering the small volume of the cisternae with a relatively large surface area where cargo proteins can interact with the glycosylation enzymes. The general morphological features of the cisternae include the following: (i) All cisternae are fenestrated; the exis-

tence of Golgi cisternae without these fenestrae has yet to be demonstrated, at least in mammalian cells; (ii) There is a *cis*-to-medial gradient of cisternae fenestration, whereby fenestrae become smaller in the medial cisternae [25]. Then, in a *trans*-wise direction, the cisternae become more perforated again, while the secretory material accumulates in nodular swellings separated by highly perforated or tubular portions [29]; (iii) The large openings in cisternae can often form 'wells'. Usually, such wells are filled with vesicles and exposed to both the *cis* and lateral sides of the stacks [25]; (iv) All Golgi cisternae have about the same surface area, but they can differ in volume by as much as 50%. The *trans*-most cisterna produces exclusively clathrin-coated buds, whereas the other cisternae display only non-clathrin-coated buds [25].

For many years, the Golgi cisternae were thought to differ from each other in their enzyme composition, and to roughly correspond to successive biosynthetic compartments. Quantitative IEM clearly demonstrates that the glycosylation enzymes are not restricted to one or two cisternae, but rather they exhibit a gradient of concentration through all the cisternae of the stack [48]. A significant difference in the distribution of Golgi enzymes can thus be detected only between the *cis*-most and *trans*-most cisternae of the central Golgi domain, and not between adjacent cisternae of the same stack [48]. Similarly, studies of glycosylation in the Golgi show that sugar nucleotide transporters and many glycosyltransferases are located in a single compartment [49]. Our view is that each single cisterna does not necessarily represent a separated Golgi compartment from either the biochemical or the structural point of view.

Interestingly, gradients of enzyme distributions have been observed not only in the *cis*-to-*trans* direction of Golgi stacks; sometimes the Golgi ribbon exhibits a gradient of enzyme concentrations along the same cisterna. Man I [our unpublished data] and Man II [50] have been seen to be at higher concentrations within the rims of cisternae and in some peri-Golgi tubules. On the other hand, the level of expansion of Golgi enzymes into tubules emerging from the cisternae might be different for different enzymes and across different cell types. For example, in RBL cells Man II has been detected in tubular structures within 200 nm of the cisternae rims. In contrast, in human fibroblasts GalTf has not been seen in membrane tubules and appears to be almost totally restricted to the cisternae profiles [our unpublished observations].

Vesicles

Vesicles of smaller (50–60 nm) and larger (up to 80–90 nm) diameters are considered to be important elements of the Golgi [26], and apparently depend on the activity of either the COPI or the clathrin machinery, respectively. However, 50–60-nm vesicles do not appear to be really 'free' because most of them are unmistakably tethered to neighboring vesicles and/or to the Golgi membranes. This explains why vesicles do not diffuse towards the cell periphery. In contrast, most of the clathrin-positive and clathrin-negative, irregularly shaped, 'vesicles' are clustered away from the Golgi [26]. Morover, many of the so-called small vesicular profiles flanking medial cisternae of the Golgi stacks in reality appear to be cross-sections through tubules extending from the rims of the these cisternae (see Morre and Keenan [51], and references therein). Finally, it is still not known whether COPI-coated (with a dense visible coat) vesicles exist. The lacelike coat found after rapid-freezing/cryosubstitution and EMT on virtual sections of 50–60 nm vesicles does not appear to be the same as the dense COPI coat found on COPI-coated buds [25]. Importantly, the same group has also reported that 50–60-nm vesicles with a lacelike coat could not be found in the other cell types [26].

The functional role of 50–60-nm vesicles

Vesicles of 50–60 nm in diameter (apparently COPI dependent) are thought to be involved either in anterograde, intra-Golgi transport of cargo [52] or in the recycling of Golgi enzymes [37]. Now it has become clear that the model that proposes a role for COPI vesicles as carriers moving cargo through the Golgi stack is inconsistent with the majority of experimental data showing the absence of cargo proteins within such vesicles [7]. The involvement of COPI vesicles in anterograde, intra-Golgi transport in vivo has been suggested on the basis of double immunogold labeling of cryosections [52]. Orci et al. [52] have demonstrated the presence of two populations of COPI vesicles; one contains anterograde cargo (proinsulin), while the other contains retrograde cargo (KDELR). However, in this report the structures considered to be vesicles might actually represent cross-sections of Golgi tubules coated with coatomer. Also, the calculations of the proinsulin concentrations within the vesicles where it is found do not take into account the level of colloidal gold penetration into the cryosections and the thickness of the cryosections. Furthermore, strong evidence against a role for 50–60-nm peri-Golgi vesicles as anterograde carriers has been published more recently [50, 53].

On the other hand, if COPI vesicles are involved in Golgi enzyme recycling (as suggested by the cisternal-maturation model of intra-Golgi transport [37]), COPI deletion should induce a shift of Golgi enzymes to the distal (post-Golgi) compartments [54]. However, in yeast [55] and in animal cells [56] COPI mutants do not appear to affect the localization of Golgi enzymes. Nonetheless, some evidence suggesting a role for COPI vesicles in retrograde transport of Golgi enzymes has also been produced recently. 'Vesicle-like' Golgi membranes containing high concentrations of enzymes have been isolated from rat liver [57, 58]. However, such vesicular membranes were not able to fuse with Golgi cisternae [59]. A recent IEM

study [50] has suggested that round profiles (possibly sections of vesicles) flanking the rims of cisternae can contain the Golgi enzyme Man II. Unfortunately, these observations are not conclusive for several reasons: First, the labeling density of Man II in peri-Golgi round profiles located within 200 nm of the cisternae rims [50] was almost the same as in the Golgi cisternae themselves. Second, others have been unable to confirm these observations [60, 61]. Orci et al. [60] have demonstrated that in comparision with the cisternae of the Golgi, the densities of Golgi enzymes (NAGTI and Man II) in all the round profiles surrounding the Golgi is 10–12-fold lower. Similarly, but more recently, the same group [61] used the same cells and antibodies as Martinez-Menarguez et al. [50] and reported a 3.6-fold exclusion of Golgi enzyme from round profiles located within a 200-nm distance from the cisternae rims. Moreover, in the vast majority of IEM studies based on cryosectioning, post-embedding or immunoperoxidase pre-embedding techniques, the density of labeling on cisternae is much higher than over the nearby round profiles [60–62]. This does not fit the prediction of the cisternal-maturation model that suggests the concentrating of Golgi enzymes within retrograde COPI vesicles [63]. Third, the Man-II-containing profiles seen at the rims of the Golgi cisternae have not yet been shown to represent vesicles rather than cross-sections of tubules or perforated cisternal rims. Finally, only one of the many Golgi enzymes, Man II, has been found in 60-nm peri-Golgi round profiles in vivo in amounts compatible with those in cisternae [50]. Thus, to our knowledge, convincing evidence in favor of Golgi enzyme concentration in COPI vesicles has not been reported to date, and therefore other Golgi enzymes and Golgi resident proteins should be studied in vivo before any general conclusions can be drawn.

Mechanisms controlling the geometry of the Golgi

The shape of the Golgi is so particular that one of the more important questions is what mechanisms control the geometry of the Golgi elements, namely: Why is there a defined number of cisternae in Golgi stacks for each cell type? Why are the cisternae so narrow? What is the role of cisternal fenestrations? Why are all the cisternae so straight and typically equal in length? and finally, What is the role of COPI-dependent vesicles? We would like to stress that transformations between cisternae, tubules and vesicles can take place very rapidly (within seconds to minutes), indicating that they must exist in a dynamic equilibrium. A well-known example is the rapid and reversible tubulation of the Golgi cisternae induced by the fungal toxin brefeldin A (BFA) [64], although fast changes in membrane shape and curvature occur also during normal intracellular traffic. Curvature changes are necessarily coupled to large modifications in transmembrane surface asymmetry [65]. These processes might also be multiple and imply the modification of membrane lipid composition (reviewed in Corda et al. [66]) or of transmembrane lipid transport by flippases [67, 68]. The other proteins so far credited with having an important role in controlling these rapid changes in morphology of the cisternae belong to the ARF/COPI machinery [55, 69], although the precise mechanism of their action is not yet understood.

A further factor which might define the geometry of cisternae is the pH within the lumen of the Golgi membranes. Both the pH [70] and the thickness of the cisternae [29] progressively decrease in the *cis*-to-*trans* direction through the Golgi stack. Thus a low pH might be responsible for the reduction of cisternae thickness due to the ability of many cargo proteins to undergo self-aggregation in an acidic enviroment [70, 71]. Such aggregation can induce a decrease in osmotic pressure in the lumen of the Golgi cisternae. As a result, water leaves the cisterna lumen, inducing its narrowing. Indeed, conversely, substances increasing the lumenal pH (NH_4Cl, bafilomycin, and so on) induce swelling of the Golgi cisternae [72].

Connections between heterotypic cisternae

Recent analyses of the 3D organization of the Golgi in fast-frozen cells (which excludes the possibility of fixation artifacts) have not revealed any completely isolated Golgi cisternae [25, 26, 73]. Thus, the idea that the whole Golgi might represent a united membranous system [22, 23, 27], where all stacks are connected with each other, cannot be neglected. Furthermore, not only all stacks, but also all the cisternae might be connected to each other (either constantly or transiently) by tubules or direct cisterna-to-cisterna contact. Tubules might also be involved in interconnections between different Golgi compartments, thus providing the physical substrate for continuity-based traffic (fig. 3). Membrane bridges have been reported to occur not only between homotypic, but also between heterotypic Golgi cisternae of adjacent stacks [23, 74, 75]. In contrast, EMT has revealed no connections between cisternae located at different levels of the adjacent stack [25]. However, this last study was limited to only a

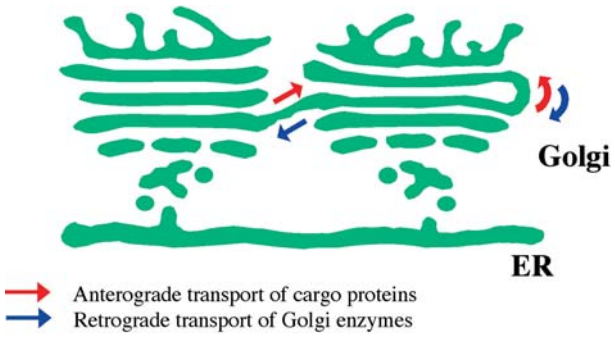

Figure 3. Potential role of intercisternae connections in the process of intra-Golgi transport.

small portion of the entire Golgi ribbon and does not operate with immunolabeling for the recognition of cisternae with different molecular compositions.

As previously indicated [41], whether or not heterotypic Golgi cisternae can be connected is still an issue of ongoing debate, the settling of which will be important towards the full understanding of how intra-Golgi transport occurs. Such membrane connections between neighboring stacks have been suggested to play a role in the transfer of proteins from the *cis*- to the *trans*-Golgi compartments [41]. However, this model also faces serious challenges from some of the experimental data. First, several cargo molecules (e.g. procollagen I) cannot diffuse through the Golgi [our unpublished observation]. Second, in nocodazole-treated cells, the Golgi mini-stacks are not connected in the normal ribbonlike fashion, but they still support transport at the normal rate [44]. However, we cannot exclude the possibility that different cisternae within each single mini-stack might be connected to each other. Moreover, if some large proteins (like procollagen I) cannot diffuse, tubular connections might be used to transport Golgi enzymes back towards this cargo and, therefore, provide its glycosylation and progression through the stack.

Nevertheless, many questions regarding the nature of the membrane connections within Golgi stacks remain unclear. We need to understand whether such connections exist stably or appear only transiently, whether they depend on the amount of cargo loaded into the Golgi and, finally, what molecular mechanisms are involved in their regulation.

The *trans*-Golgi

The *trans*-face of the Golgi has attracted the attention of cell biologists since its identification as the exit pole of the Golgi complex, where transport proteins are directed to their final destinations. To understand the mechanisms of sorting and exit from the Golgi complex, it has been essential to identify the specific compartment in which these processes take place. This compartment is now defined as the TGN, although its precise organization still remains a point of debate.

The structure of the TGN

Biochemical and IEM studies have revealed that the TGN is involved in the terminal glycosylation of proteins as well as in cargo packaging into membrane carriers destined for the PM or the endosomal/lysosomal system [8]. Most of the information concerning TGN morphology has come from the EM studies published by the groups of Rambourg [76] and Griffiths [10, 77]. The TGN has generally been considered to be the *trans*-most cisterna of the Golgi stack, which is also seen to continue into the large anastomoting tubular network [10, 76, 77]. In contrast, some reports have considered the TGN as an entirely independent organelle [78, 79]. Indeed, in some cell types, the purely tubulated TGN is positioned at some distance from the *trans* aspect of the Golgi ribbon [76]. On the other hand, in many cell types, connections between the TGN and the stack of cisternae can be easily visualized [10, 76, 77].

Analyses of different cell types have revealed that the TGN can vary significantly in both size and composition. For example, cells without secretory granules exhibit an extensive tubular TGN, while the tubular component of the TGN is reduced in cells specialized in regulated secretion [76]. This appears to occur because in the latter, most of the TGN membranes are used for the packaging of secretory granules, and tubules only form thin bridges between the granule precursors. Such an organization simplifies the release of the granules, which occurs through the rupture of these tubular elements [76]. Thus, the morphology and size of the TGN apparently depend on the predominant type and amount of cargo protein departing from the Golgi complex. Indeed, when the exit from the Golgi is blocked by a lowering of the temperature to 20 °C, the volume and surface area of the TGN is seen to increase [10].

An alternative view of the organization at the *trans* face of the Golgi comes from studies using EMT of cryofixed, freeze-substituted cells [25]. Surprisingly, the authors demonstrated a lack of the classical TGN. Instead, the three *trans*-most cisternae exhibited tubules that frequently contained coat on their tips, and that extended into the *trans* space of the Golgi stacks. Each of these cisternae appeared to produce only one type of coated vesicular structure, with only the *trans*-most cisterna having clathrin-coated buds. Thus, this study suggests that the three *trans*-most cisternae of the Golgi stacks serve the role of the classical TGN, and that each of them is specialized in the packaging and export of its own, specific, cargo proteins. However, more recent studies by the same authors have shown that upon a 20 °C block, all three of these *trans*-most cisternae produced bulging exit domains with a similar morphology [73]. Therefore, it is not clear whether the last of these three *trans*-most cisternae is only specialized in the formation of clathrin-coated carriers, or whether it also exports cargo through the bulging domains in a fashion similar to that seen for the other two *trans* cisternae. Unfortunately, the lack of immunolabeling in this study does not allow it to be said whether different *trans* cisternae really do contain different transport proteins. Indeed, the cargo protein VSVG does not appear to be excluded from any of the *trans* cisternae during its exit from the Golgi [10, 77, 80]. Therefore, the possibility that a TGN compartment with a tubular-reticular morphology exists and performs the sorting of the cargo proteins can definitely not be excluded.

Although the *trans*-most cisterna can often be seen to 'peel off' from the rest of the Golgi stack [76] or even to be partially separated from it by ER or vesicles [73], whether or not this last cisterna belongs to the TGN is not clear. Griffiths et al. consider it as a saccular (cisternal) part of the TGN [77]. Indeed, TGN markers, such as TGN46, are found there, although to a lesser extent in comparison with the tubular part of the TGN. However, the last cisterna also contains the Golgi-resident enzyme GalTf, which is usually excluded from the tubular-reticular portion of the TGN and is considered as a marker of the central (stacked) Golgi domains [81]. On the other hand, due to the numerous perforations, the *trans*-most cisterna frequently looks like a flat membrane web which can be transformed into the tubular-reticular part of the TGN by a few fission events [76]. Thus, the *trans*-most cisterna connected to the TGN is likely to represent a transitional element between the core of the Golgi stack and the TGN.

We would propose that these structural changes at the *trans* face of the Golgi are related to the ability of the TGN to sort various cargo proteins from the resident components of the Golgi stack. Before exit from the Golgi, the cargo proteins leave behind molecules, such as the glycosylation enzymes, that reside within the core region of the Golgi stack [80, 82]. This event might be crucial for the transition of the Golgi membranes from a cisterna-like morphology to the tubular-reticular morphology. This probably happens because several Golgi enzymes tend to form large oligomers via kin recognition, and therefore stabilize the flat shape of the cisterna [83]. When cargo arrives at the TGN, it has already acquired all the necessary glycosylation and can finally lose its association with the Golgi enzymes, which might stabilize the flat shape of the cisternae. Thus, the TGN membranes devoid of the Golgi enzymes should be prone to undergoing tubulation.

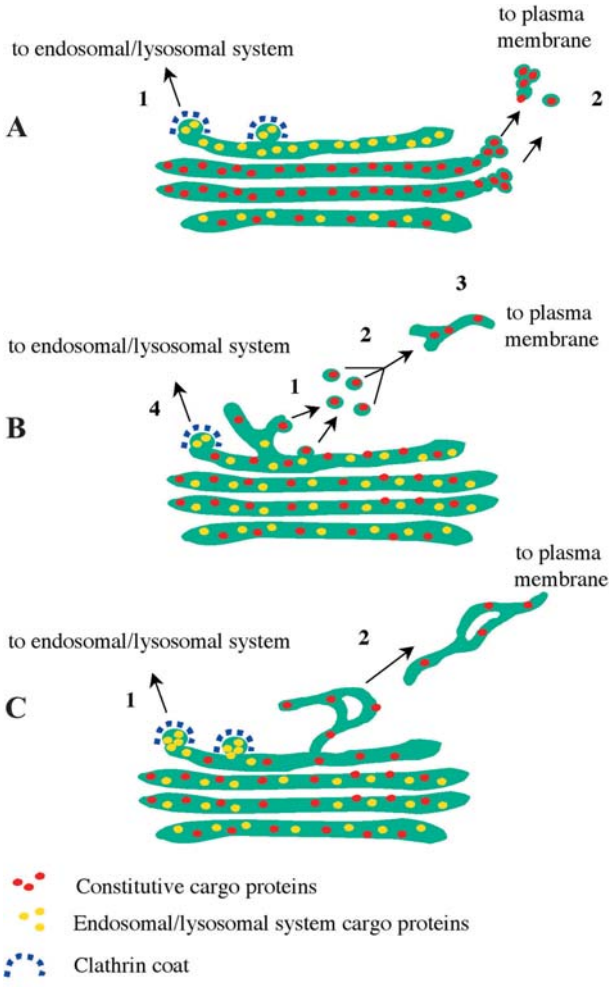

Figure 4. Possible mechanisms of cargo export from the Golgi complex. (*A*) The *trans*-most cisterna of the Golgi stack contains clathrin-coated buds and serves in the exit of the endosomal-lysosomal cargo (1), while the other two *trans* cisternae provide the export of the constitutive cargo proteins (2). (*B*) Constitutive cargo proteins exit the TGN within small vesicles (1) which undergo homotypic fusion (2) into a large cargo container (3). Cargo directed to the endosomal-lysosomal system is sorted at the TGM into clathrin-coated domains and carriers (4). (*C*) Endosomal-lysosomal cargo is sorted into clathrin-coated domains and carriers (1), while constitutive cargo proteins remain in the bulk of the TGN membranes and then exit the Golgi through the fission of complex tubular-reticular domains from the rest of the TGN (2).

The mechanism of formation of post-Golgi carriers

Various cargo proteins need to be moved within the TGN to the different exit domains where the transport carriers are formed. PM-directed proteins, such as VSVG, leave the Golgi within Golgi-to-PM carriers (GPCs), which at the EM level have a complex, tubular-saccular morphology [80]. Some of these GPCs even exhibit fenestrae, which until recently was thought to be a feature exclusive to Golgi membranes. How such complex structures as GPCs emerge from the Golgi was unclear until recently (see fig. 4 for the various models under discussion at present). They might form from the bulging domains of the multiple *trans* cisternae of the stacks (fig. 4A), as has been suggested by Ladinsky et al. [73]. On the other hand, GPC biogenesis might imply fusion of small, TGN-derived vesicles into larger structures (fig. 4B); likewise, the formation of EGCs is thought to require fusion of COPII vesicles. However, our recent work suggests that GPCs do not arise as a result of vesicle fusion [84]. In contrast, they form by fission of complex tubular-reticular membranes from specialized areas of the TGN (GPC precursors) which extrude from the Golgi along MTs using kinesin motors (fig. 4C). The GPC precursors do not concentrate VSVG, and remain in physical continuity with the TGN membranes. At the same time, GPCs exclude other TGN proteins directed to the endosomal/lysosomal system, such as M6PR, lamp-1 and furin, indicating that a segregation process does take place. How can these observations be rationalized in a simple scheme?

Apparently cargoes directed to the endosomes, such as MPR 6 and furin, segregate into relatively small TGN domains in which they undergo coat-mediated concentration before exit. For instance, recruitment of AP-1 to TGN membranes drives a strong concentrating of M6PR [85, 86]. Similarly, other cargoes, such as furin [87] and lamp-1 [88], tend to leave the bulk TGN for relatively small clathrin-coated domains (see scheme in fig. 4C). Instead, the constitutive cargo protein VSVG resides throughout the entire TGN structure, with the exception of the clathrin-coated buds [77, 84], and does not undergo concentration to any significant extent (fig. 4C). The exclusion of VSVG from such buds does not result in a detectably higher VSVG level in GPC precursors versus the rest of the TGN because of the small volume of these buds from which VSVG is kept out. Therefore, VSVG apparently exits within large blocks of Golgi membranes by a bulk-flow-like mechanism.

Exit from the Golgi towards the endocytic compartments

Given the above context, what is the role of coat proteins in export from the TGN? Recent data indicate that clathrin, AP-1, AP-3, AP-4 (adaptor proteins) and the GGAs are excluded from GPC precursors [84]. This is in line with previous reports showing the absence of coat and APs on already formed carriers [80, 82]. Moreover, blockade of the AP-1, AP-3 and clathrin-budding machineries has been reported as not affecting the secretion of constitutive cargo proteins, while it interferes with the regular trafficking of lysosomal proteins [89–91]. In living cells, M6PR appears to leave the Golgi in carriers of variable sizes coated with GGAs [92]. Thus the APs and coats are apparently required for the exit of cargo targeted to compartments of the endosomal system. On the other hand, the AP-1B complex has been shown to play an important role in the correct targeting of cargo proteins from the Golgi to the basolateral portion of the PM [93]. However, AP-1B is expressed only in polarized epithelial cells [94], and it is not yet clear which adaptor substitutes for it in other cell types.

Finally, a possible role for coat proteins in TGN export might be in recruiting machinery proteins to different cargo exit domains, such as the appropriate SNARE and kinesin isoforms. Indeed, coat-mediated lipid and protein partitioning at the TGN might lead to preferential binding of various kinesins to different membrane domains. For example, KIF13A has been shown to interact with AP1 and to participate in the transport of M6PR [95], while KIFC3 has been shown to be associated with cholesterol-rich membranes and to drive the delivery of cargo proteins to the apical membrane of polarized MDCK cells [96]. Recently, we demonstrated that the TGN sites of GPC assembly can be labeled with an antibody against conventional kinesin [84], which is thought to be involved in budding and transport of constitutive carriers from the TGN [97]. Thus, selective recruitment of the correct motor proteins to different TGN subdomains can provide a high-fidelity, motor-dependent exit of different cargo proteins from the TGN, and their delivery to the target compartments.

Cell polarity and exit from the Golgi

According to the current consensus, apical/basolateral sorting of proteins and lipids occurs at the level of the TGN [8]. Therefore, the proteins with apical or basolateral sorting signals are thought to be packed directly from the TGN into different transport carriers which deliver them to the correct PM regions of polarized cells [98, 99]. However, instead of this TGN sorting, many polarized cells rely heavily on transcytosis to direct proteins to the apical surface [100, 101]. Thus it is not clear whether all cells have a specialized domain of the TGN for proteins with apical sorting signals, or what might be the ultrastructure of this domain. Some molecules, such as MAL [102] and caveolin [103], have been proposed to play a role as adaptors in order to drive the segregation of apical cargoes within the TGN. However, both of these proteins do not drive the formation of specific structures at the TGN, where they reside in coatless membrane domains [102, 104]. Moreover, and as with VSVG [80], apical markers have been found in the tubular domains of the TGN and in the pleiomorphic GPCs [102]. On the other hand, the basolateral sorting signals of the membrane proteins described to date reside in their cytoplasmic domains, and they require an interaction with an adaptor (for review, see Nelson and Yeaman [105]). However, whether these interactions occur directly at the TGN or within more distal endocytic compartments is an issue of ongoing debate [93, 106].

Therefore, if apical/basolateral segregation in the TGN exists, it might rely more on spontaneous partitioning of the proteins into domains with different lipid compositions rather then on the coat and adaptor proteins. Indeed, apically targeted GPI-anchored proteins, as well as transmembrane proteins (like the hemagglutinin of the influenza virus), are able to associate with cholesterol-rich lipid rafts [107]. Thus, apical and basolateral cargo could be distributed to the different domains of the lipid mosaic at the TGN, and in this way sorted into different carriers. However, several reports indicate that partitioning into the lipid rafts is not an essential prerequisite for apical delivery of proteins [108]. Moreover, colocalization of GPI-anchored and basolateral proteins has recently been detected in GPCs [109]. Thus, apical/basolateral sorting at the TGN level appears to be a rather stochastic process and to require further efforts to be understood in full.

The mechanisms of Golgi-to-apical-PM transport could actually be defined by relocation of the Golgi towards the apical-PM domain during polarization of the cells (see

also above). In polarized cells, the Golgi forms a cap surrounding the centrosome and facing the apical portion of the PM [17]. When polarized enterocytes are treated with nocodazole to depolymerize microtubules, additional apical domains form along their basolateral cell surface. Interestingly, Golgi fragments appear to be always associated with these new apical portions of the PM [M. Pavelka, personal communication]. What is the mechanism responsible for such an association? One possibility could be an interaction of the Golgi membranes with actin, which is concentrated in the subapical space of polarized cells [110]. In addition, a KIFC3 motor has been reported to play a role in both the delivery of proteins to the apical PM [96] and the correct positioning of the Golgi to the centrosome [18]; therefore, this might also provide a link between the Golgi and the apical surface in epithelial cells.

Cargo as a modulator of Golgi structure and function

Several lines of evidence have suggested a role of cargo as a potent determinant of Golgi structure and function. For example, in yeast, a 3-h treatment with cycloheximide leads to the complete disappearance of the Golgi [111]. Also, in the primitive eukaryote *Giardia lamblia*, the typical Golgi is absent during the proliferative stage, when secretion is quite limited. However, the Golgi stacks reappear when the cell begins to secrete glycans needed for cyst formation [112]. It is not clear, however, whether in *G. lamblia* the Golgi glycosidases appear first, or whether the synthesis of the proteins with glycosylation sites induces the expression of the Golgi enzymes. Similarly, the extent of cargo influx influences the organization of the mammalian Golgi. Here, blockade of protein synthesis with cycloheximide leads to the formation of very thin cisternae in onion-like Golgi stacks [113]. Moreover, when cargo proteins within the ER are arrested by transfection of a Sar1p dominant-negative mutant [43], all Golgi structure is lost within 12–18 h. In contrast, when cargo influx increases sharply (e.g. 5 min after ER-transport-block release), the Golgi immediately grows in size [114]. Thus, apparently even in mammalian cells the Golgi tends to disappear gradually in the absence of cargo input, while it becomes larger when transport intensifies. This phenomenon has been clearly illustrated by the stimulation and inhibition of prolactin transport in lactating rats [115]. Two hours after the blockage of hormonal stimulation of prolactin-producing cells the CGN disappears from the stacks. Simultaneously, the Golgi ribbon undergoes fragmentation at the level of the mid-compartment. Furthermore, 12 h later, the size of the Golgi reduces dramatically, and no secretory granules are visible [115]. If the stimulation is then restored, the CGN reappears in 20 min. The poorly perforated cisternae increase in size [115]. Interestingly, the number of small Golgi vesicles increases when the Golgi regresses, and decreases when the Golgi is stimulated to produce secretory granules [115]. Thus, the vesicles might play an important role in the transformation of the Golgi structure in response to the changing cargo influx.

However, many issues regarding the links between intensity of transport and Golgi structure still remain obscure. Is there partitioning into different compartments by the Golgi enzymes and cargo during intra-Golgi transport? What molecular machineries are recruited to the Golgi upon stimulation of transport, and to what extent? These questions represent new challenges that now need to be faced.

Future perspectives

We hope that this tour through the Golgi with a focus on the structural aspects of traffic has not only provided a glimpse of a novel vision of Golgi structure and intracellular transport, but has also provided an awareness of the large knowledge gaps. Indeed, our analysis of the morpho-functional organization of the Golgi has revealed many uncertainties and unclear questions. Most of these are based on the consideration that Golgi structure and function are tightly interconnected. While humbling, this overview should, however, suggest that the task of completing the picture is no longer as daunting as it appeared until a few years ago, particularly as more technological and conceptual tools are now available to resolve these issues. We hope that with the help of these tools the mechanisms of protein transfer though the Golgi will finally be unraveled, and that the molecular machineries regulating this process will be placed in their correct locations within this, our favorite, organelle.

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1 Palade G. (1975) Intracellular aspects of the process of protein synthesis. Science **189:** 347–358
2 Antonny B. and Schekman R. (2001) ER export: public transportation by the COPII coach. Curr. Opin. Cell Biol. **13:** 438–443
3 Nickel W., Brugger B. and Wieland F. T. (2002) Vesicular transport: the core machinery of COPI recruitment and budding. J. Cell Sci. **115:** 3235–3240
4 Mayer A. (2002) Membrane fusion in eukaryotic cells. Annu. Rev. Cell Dev. Biol. **18:** 289–314

5 Rothman J. E. and Wieland F. T. (1996) Protein sorting by transport vesicles. Science **272:** 227–234
6 Beznoussenko G. V. and Mironov A. A. (2002) Models of intracellular transport and evolution of the Golgi complex. Anat. Rec. **268:** 226–238
7 Storrie B. and Nilsson T. (2002) The Golgi apparatus: balancing new with old. Traffic **3:** 521–529
8 Keller P. and Simons K. (1997) Post-Golgi biosynthetic trafficking. J. Cell Sci. **110:** 3001–3009
9 Roth J. and Berger E. G. (1982) Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. J. Cell Biol. **93:** 223–229
10 Griffiths G., Fuller S. D., Back R., Hollinshead M., Pfeiffer S. and Simons K. (1989) The dynamic nature of the Golgi complex. J. Cell Biol. **108:** 277–297
11 Kornfeld R. and Kornfeld S. (1985) Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **54:** 631–664
12 Schafer W., Stroh A., Berghofer S., Seiler J., Vey M., Kruse M. L. et al. (2002) Two independent targeting signals in the cytoplasmic domain determine trans-Golgi network localization and endosomal trafficking of the proprotein convertase furin. EMBO J. **14:** 2424–2435
13 Thyberg J. and Moskalewski S. (1985) Microtubules and the organization of the Golgi complex. Exp. Cell Res. **159:** 1–16
14 Ho W. C., Allan V. J., van Meer G., Berger E. G. and Kreis T. E. (1989) Reclustering of scattered Golgi elements occurs along microtubules. Eur. J. Cell Biol. **48:** 250–263
15 Kupfer A., Louvard D. and Singer S. J. (1982) Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound. Proc. Natl. Acad. Sci. USA **79:** 2603–2607
16 Kupfer A., Dennert G. and Singer S. J. (1983) Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. Proc. Natl. Acad. Sci. USA **80:** 7224–7228
17 Ojakian G. K., Nelson W. J. and Beck K. A. (1997) Mechanisms for de novo biogenesis of an apical membrane compartment in groups of simple epithelial cells surrounded by extracellular matrix. J. Cell Sci. **110:** 2781–2794
18 Xu Y., Takeda S., Nakata T., Noda Y., Tanaka Y. and Hirokawa N. (2002) Role of KIFC3 motor protein in Golgi positioning and integration. J. Cell Biol. **158:** 293–303
19 Rambourg A. and Clermont Y. (1990) Three-dimensional electron microscopy: structure of the Golgi apparatus. Eur. J. Cell Biol. **51:** 189–200
20 Farquhar M. G. and Palade G. E. (1981) The Golgi apparatus (complex) – (1954–1981) – from artifact to center stage. J. Cell Biol. **91:** 77s–103s
21 Sasaki T. (1983) Ultrastructure and cytochemistry of the Golgi apparatus and related organelles of the secretory ameloblasts of the rat incisor. Arch. Oral. Biol. **28:** 895–905
22 Tanaka K., Mitsushima A., Fukudome H. and Kashima Y. (1986) Three-dimensional architecture of the Golgi complex observed by high resolution scanning electron microscopy. J. Submicrosc. Cytol. **18:** 1–9
23 Clermont Y., Rambourg A. and Hermo L. (1994) Connections between the various elements of the cis- and mid-compartments of the Golgi apparatus of early rat spermatids. Anat. Rec. **240:** 469–480
24 Dylewski D. P., Haralick R. M. and Keenan T. W. (1984) Three-dimensional ultrastructure of the Golgi apparatus in bovine mammary epithelial cells during lactation. J. Ultrastruct. Res. **87:** 75–85
25 Ladinsky M. S., Mastronarde D. N., McIntosh J. R., Howell K. E. and Staehelin L.A. (1999) Golgi structure in three dimensions: functional insights from the normal rat kidney cell. J. Cell Biol. **144:** 1135–1149
26 Marsh B. J., Mastronarde D. N., Buttle K. F., Howell K. E. and McIntosh J. R. (2001) Organellar relationships in the Golgi region of the pancreatic beta cell line, HIT-T15, visualized by high resolution electron tomography. Proc. Natl. Acad. Sci. USA **98:** 2399–2406
27 Cole N. B., Smith C. L., Sciaky N., Terasaki M., Edidin M. and Lippincott-Schwartz J. (1996) Diffusional mobility of Golgi proteins in membranes of living cells. Science **273:** 797–801
28 Mellman I. and Simons K. (1992) The Golgi complex: in vitro veritas? Cell **68:** 829–840
29 Rambourg A. and Clermont Y. (1997) Three-dimensional structure of the Golgi apparatus in mammalian cells. In: The Golgi apparatus, Berger E. G. and Roth J. (eds), Birkhäuser, Basel
30 Orci L., Montesano R., Meda P., Malaisse-Lagae F., Brown D., Perrelet A. et al. (1981) Heterogeneous distribution of filipin – cholesterol complexes across the cisternae of the Golgi apparatus. Proc. Natl. Acad. Sci. USA **78:** 293–297
31 Cluett E. B., Kuismanen E. and Machamer C. E. (1997) Heterogeneous distribution of the unusual phospholipid semilysobisphosphatidic acid through the Golgi complex. Mol. Biol. Cell **8:** 2233–2240
32 Mobius W., Ohno-Iwashita Y., van Donselaar E. G., Oorschot V. M., Shimada Y., Fujimoto T. et al. (2002) Immunoelectron microscopic localization of cholesterol using biotinylated and non-cytolytic perfringolysin O. J. Histochem. Cytochem. **50:** 43–55
33 Sciaky N., Presley J., Smith C., Zaal K. J., Cole N., Moreira J. E. et al. (1997) Golgi tubule traffic and the effects of brefeldin A visualized in living cells. J. Cell Biol. **139:** 1137–1155
34 White J., Johannes L., Mallard F., Girod A., Grill S., Reinsch S. et al. (1999) Rab6 coordinates a novel Golgi to ER retrograde transport pathway in live cells. J. Cell Biol. **147:** 743–760
35 Pelham H. R. (1997) Membrane transport. Green light for Golgi traffic. Nature **389:** 17–19
36 Presley J. F., Cole N. B., Schroer T. A., Hirschberg K., Zaal K. J. and Lippincott-Schwartz J. (1997) ER-to-Golgi transport visualized in living cells. Nature **389:** 81–85
37 Glick B. S. and Malhotra V. (1998) The curious status of the Golgi apparatus. Cell **95:** 883–889
38 Marra P., Maffucci T., Daniele T., Di Tullio G., Ikehara Y., Chan E. K. et al. (2001) The GM130 and GRASP65 Golgi proteins cycle through and define a subdomain of the intermediate compartment. Nat. Cell Biol. **3:** 1101–1113
39 Ayala J. (1994) Transport and internal organization of membranes: vesicles, membrane networks and GTP-binding proteins. J. Cell Sci. **107:** 753–763
40 Novikoff P. M., Novikoff A. B., Quintana N. and Hauw J. J. (1971) Golgi apparatus, GERL, and lysosomes of neurons in rat dorsal root ganglia, studied by thick section and thin section cytochemistry. J. Cell Biol. 50: 859–886
41 Mironov A. A., Weidman P. and Luini A. (1997) Variations on the intracellular transport theme: maturing cisternae and trafficking tubules. J. Cell Biol. **138:** 481–484
42 Aridor M., Fish K. N., Bannykh S., Weissman J., Roberts T. H., Lippincott-Schwartz J. et al. (2001) The Sar1 GTPase coordinates biosynthetic cargo selection with endoplasmic reticulum export site assembly. J. Cell Biol. **152:** 213–229
43 Ward T. H., Polishchuk R. S., Caplan S., Hirschberg K. and Lippincott-Schwartz J. (2001) Maintenance of Golgi structure and function depends on the integrity of ER export. J. Cell Biol. **155:** 557–570
44 Cole N. B., Sciaky N., Marotta A., Song J. and Lippincott-Schwartz J. (1996) Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. Mol. Biol. Cell **7:** 631–650
45 Polishchuk R. S., Polishchuk E. V. and Mironov A. A. (1999) Coalescence of Golgi fragments in microtubule-deprived living cells. Eur. J. Cell Biol. **78:** 170–185

46 Storrie B., White J., Rottger S., Stelzer E. H., Suganuma T. and Nilsson T. (1998) Recycling of golgi-resident glycosyltransferases through the ER reveals a novel pathway and provides an explanation for nocodazole-induced Golgi scattering. J. Cell Biol. **143:** 1505–1521

47 Griffiths G. (2000) Gut thoughts on the Golgi complex. Traffic **1:** 738–745

48 Rabouille C., Hui N., Hunte F., Kieckbusch R., Berger E. G., Warren G. et al. (1995) Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. J. Cell Sci. **108:** 1617–1627

49 Varki A. (1998) Factors controlling the glycosylation potential of the Golgi apparatus. Trends Cell Biol. **8:** 34–40

50 Martinez-Menarguez J. A., Prekeris R., Oorschot V. M., Scheller R., Slot J. W., Geuze H. J. et al. (2001) Peri-Golgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. J. Cell Biol. **155:** 1213–1224

51 Morre D. J. and Keenan T. W. (1994). Golgi apparatus buds – vesicles or coated ends of tubules? Protoplasma **179:** 1–4

52 Orci L., Stamnes M., Ravazzola M., Amherdt M., Perrelet A., Sollner T. H. et al. (1997) Bidirectional transport by distinct populations of COPI-coated vesicles. Cell **90:** 335–349

53 Mironov A. A., Beznoussenko G. V., Nicoziani P., Martella O., Trucco A., Kweon H. S. et al. (2001) Small cargo proteins and large aggregates can traverse the Golgi by a common mechanism without leaving the lumen of cisternae. J. Cell Biol. **155:** 1225–1238

54 Nickel W. and Wieland F. T. (1998) Biosynthetic protein transport through the early secretory pathway. Histochem. Cell Biol. **109:** 477–486

55 Gaynor E. C., Graham T. R. and Emr S. D. (1998) COPI in ER/Golgi and intra-Golgi transport: do yeast COPI mutants point the way? Biochim. Biophys. Acta **1404:** 33–51

56 Guo Q., Vasile E. and Krieger M. (1994) Disruptions in Golgi structure and membrane traffic in a conditional lethal mammalian cell mutant are corrected by epsilon-COP. J. Cell Biol. **125:** 1213–1224

57 Lanoix J., Ouwendijk J., Lin C. C., Stark A., Love H. D., Ostermann J. et al. (1999) GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles. EMBO J. **18:** 4935–4948

58 Lanoix J., Ouwendijk J., Stark A., Szafer E., Cassel D., Dejgaard K. et al. (2001) Sorting of Golgi resident proteins into different subpopulations of COPI vesicles: a role for ArfGAP1. J. Cell Biol. **155:** 1199–1212

59 Lin C. C., Love H. D., Gushue J. N., Bergeron J. J. and Ostermann J. (1999) ER/Golgi intermediates acquire Golgi enzymes by brefeldin A-sensitive retrograde transport in vitro. J. Cell Biol. **147:** 1457–1472

60 Orci L., Amherdt M., Ravazzola M., Perrelet A. and Rothman J. E. (2000) Exclusion of golgi residents from transport vesicles budding from Golgi cisternae in intact cells. J. Cell Biol. **150:** 1263–1270

61 Cosson P., Amherdt M., Rothman J. E. and Orci L. (2002) A resident Golgi protein is excluded from peri-Golgi vesicles in NRK cells. Proc. Natl. Acad. Sci. USA **99:** 12831–12834

62 Velasco A., Hendricks L., Moremen K. W., Tulsiani D. R., Touster O. and Farquhar M. G. (1993) Cell type-dependent variations in the subcellular distribution of alpha-mannosidase I and II. J. Cell Biol. **122:** 39–51

63 Glick B. S., Elston T. and Oster G. (1997) A cisternal maturation mechanism can explain the asymmetry of the Golgi stack. FEBS Lett. **414:** 177–181

64 Klausner R. D., Donaldson J. G. and Lippincott-Schwartz J. (1992) Brefeldin A: insights into the control of membrane traffic and organelle structure. J. Cell Biol. **116:** 1071–1080

65 Burger K. N. (2000) Greasing membrane fusion and fission machineries. Traffic **1:** 605–613

66 Corda D., Hidalgo Carcedo C., Bonazzi M., Luini A. and Spanò S. (2002) Molecular aspects of membrane fission in the secretory pathway. Cell. Mol. Life Sci. **59:** 1819–1832

67 Pomorski T., Hrafnsdottir S., Devaux P. F. and van Meer G. (2001) Lipid distribution and transport across cellular membranes. Semin. Cell. Dev. Biol. **12:** 139–148

68 Holthuis J. C., Pomorski T., Raggers R. J., Sprong H. and Van Meer G. (2001) The organizing potential of sphingolipids in intracellular membrane transport. Physiol. Rev. **81:** 1689–1723

69 Lippincott-Schwartz J., Cole N. B. and Donaldson J. G. (1998) Building a secretory apparatus: role of ARF1/COPI in Golgi biogenesis and maintenance. Histochem. Cell Biol. **109:** 449–462

70 Wu M. M., Grabe M., Adams S., Tsien R. Y., Moore H. P. and Machen T. E. (2001) Mechanisms of pH regulation in the regulated secretory pathway. J. Biol. Chem. **276:** 33027–33035

71 Tooze S. A. (1998) Biogenesis of secretory granules in the trans-Golgi network of neuroendocrine and endocrine cells. Biochim. Biophys. Acta **1404:** 231–244

72 Berger E. G., Berger B., Hochli M. and Dinter A. (2001) Colocalization of beta 1,4galactosyltransferase with mannose 6-phosphate receptor in monensin-induced TGN-derived structures. Histochem. Cell Biol. **115:** 157–168

73 Ladinsky M. S., Wu C. C., McIntosh S., McIntosh J. R. and Howell K. E. (2002) Structure of the Golgi and distribution of reporter molecules at 20 degrees C reveals the complexity of the exit compartments. Mol. Biol. Cell **13:** 2810–2825

74 Hermo L., Rambourg A. and Clermont Y. (1980) Three-dimensional architecture of the cortical region of the Golgi apparatus in rat spermatids. Am. J. Anat. **157:** 357–373

75 Thorne-Tjomsland G., Clermont Y. and Tang X. M. (1991) Glucose-6-phosphatase activity of endoplasmic reticulum and Golgi apparatus in spermatocytes and spermatids of the rat: an electron microscopic cytochemical study. Biol. Cell **71:** 33–41

76 Clermont Y., Rambourg A. and Hermo L. (1995) Trans-Golgi network (TGN) of different cell types: three-dimensional structural characteristics and variability. Anat. Rec. **242:** 289–301

77 Griffiths G., Pfeiffer S., Simons K. and Matlin K. (1985) Exit of newly synthesized membrane proteins from the trans cisterna of the Golgi complex to the plasma membrane. J. Cell Biol. **101:** 949–964

78 Geuze H. J. and Morre D. J.(1991) Trans-Golgi reticulum. J. Electron Microsc. Tech. **17:** 24–34

79 Reaves B. and Banting G. (1992) Perturbation of the morphology of the trans-Golgi network following Brefeldin A treatment: redistribution of a TGN-specific integral membrane protein, TGN38. J. Cell Biol. **116:** 85–94

80 Polishchuk R. S., Polishchuk E. V., Marra P., Buccione R., Alberti S., Luini A. et al. (2000) GFP-based correlative light-electron microscopy reveals the saccular-tubular ultrastructure of carriers in transit from the Golgi apparatus to the plasma membrane. J. Cell Biol. **148:** 45–58

81 Prescott A. R., Lucocq J. M., James J., Lister J. M. and Ponnambalam S. (1997) Distinct compartmentalization of TGN46 and beta 1,4-galactosyltransferase in HeLa cells. Eur. J. Cell Biol. **72:** 238–246

82 Hirschberg K., Miller C. M., Ellenberg J., Presley J. F., Siggia E. D., Phair R. D. et al. (1998) Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. J. Cell Biol. **143:** 1485–1503

83 Nilsson T., Hoe M. H., Slusarewicz P., Rabouille C., Watson R., Hunte F. et al. (1994) Kin recognition between medial Golgi enzymes in HeLa cells. EMBO J. **13:** 562–574

84 Polishchuk E. V., Di Pentima A., Polishchuk R. S. and Luini A. (2001) Characterisation of the mechanism of formation of

constituitive post-Golgi transport intertmediates. Mol. Biol. Cell **12:** 339a
85 Klumperman J., Hille A., Veenendaal T., Oorschot V., Stoorvogel W., von Figura K. et al. (1993) Differences in the endosomal distributions of the two mannose 6-phosphate receptors. J. Cell Biol. **121:** 997–1010
86 Doray B., Ghosh P., Griffith J., Geuze H. J. and Kornfeld S. (2002) Cooperation of GGAs and AP-1 in packaging MPRs at the trans-Golgi network. Science **297:** 1700–1703
87 Dittie A. S., Klumperman J. and Tooze S. A. (1999) Differential distribution of mannose-6-phosphate receptors and furin in immature secretory granules. J. Cell Sci. **112:** 3955–3966
88 Honing S., Griffith J., Geuze H. J. and Hunziker W. (1996) The tyrosine-based lysosomal targeting signal in lamp-1 mediates sorting into Golgi-derived clathrin-coated vesicles. EMBO J. **15:** 5230–5239
89 Dell'Angelica E. C., Shotelersuk V., Aguilar R. C., Gahl W. A. and Bonifacino J. S. (1999) Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. Mol. Cell **3:** 11–21
90 Rehling, P., Darsow T., Katzmann D. J. and Emr S. D. (1999) Formation of AP-3 transport intermediates requires Vps41 function. Nat. Cell Biol. **1:** 346–353
91 Hirst J., Lui W. W., Bright N. A., Totty N., Seaman M. N. and Robinson M. S. (2000) A family of proteins with gamma-adaptin and VHS domains that facilitate trafficking between the trans-Golgi network and the vacuole/lysosome. J. Cell Biol. **149:** 67–80
92 Puertollano R., Aguilar R. C., Gorshkova I., Crouch R. J. and Bonifacino J. S. (2001) Sorting of mannose 6-phosphate receptors mediated by the GGAs. Science **292:** 1712–1716
93 Folsch H., Pypaert M., Schu P. and Mellman I. (2001) Distribution and function of AP-1 clathrin adaptor complexes in polarized epithelial cells. J. Cell Biol. **152:** 595–606
94 Ohno H., Tomemori T., Nakatsu F., Okazaki Y., Aguilar R. C., Foelsch H. et al. (1999) Mu1B, a novel adaptor medium chain expressed in polarized epithelial cells. FEBS Lett. **449:** 215–220
95 Nakagawa T., Setou M., Seog D., Ogasawara K., Dohmae N., Takio K. et al. (2000) A novel motor, KIF13A, transports mannose-6-phosphate receptor to plasma membrane through direct interaction with AP-1 complex. Cell **103:** 569–581
96 Noda Y., Okada Y., Saito N., Setou M., Xu Y., Zhang Z. et al. (2001) KIFC3, a microtubule minus end-directed motor for the apical transport of annexin XIIIb-associated Triton-insoluble membranes. J. Cell Biol. **155:** 77–88
97 Kreitzer G., Marmorstein A., Okamoto P., Vallee R. and E. Rodriguez-Boulan. (2000) Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. Nat. Cell Biol. **2:** 125–127
98 Ikonen E., Tagaya M., Ullrich O., Montecucco C. and Simons K. (1995) Different requirements for NSF, SNAP, and Rab proteins in apical and basolateral transport in MDCK cells. Cell **81:** 571–580
99 Keller P., Toomre D., Diaz E., White J. and Simons K. (2001) Multicolour imaging of post-Golgi sorting and trafficking in live cells. Nat. Cell Biol. **3:** 140–149
100 Bartles J. R., Feracci H. M., Stieger B. and Hubbard A. L. (1987) Biogenesis of the rat hepatocyte plasma membrane in vivo: comparison of the pathways taken by apical and basolateral proteins using subcellular fractionation. J. Cell Biol. **105:** 1241–1251
101 Bonilha V. L., Marmorstein A. D., Cohen-Gould L. and Rodriguez-Boulan E. (1997) Apical sorting of influenza hemagglutinin by transcytosis in retinal pigment epithelium. J. Cell Sci. **110:** 1717–1727
102 Puertollano R., Martinez-Menarguez J. A., Batista A., Ballesta J. and Alonso M. A. (2001) An intact dilysine-like motif in the carboxyl terminus of MAL is required for normal apical transport of the influenza virus hemagglutinin cargo protein in epithelial Madin-Darby canine kidney cells. Mol. Biol. Cell **12:** 1869–1883
103 Zurzolo C., van't Hof W., van Meer G. and Rodriguez-Boulan E. (1994) VIP21/caveolin, glycosphingolipid clusters and the sorting of glycosylphosphatidylinositol-anchored proteins in epithelial cells. EMBO J. **13:** 42–53
104 Lipardi C., Mora R., Colomer V., Paladino S., Nitsch L., Rodriguez-Boulan E. et al. (1998) Caveolin transfection results in caveolae formation but not apical sorting of glycosylphosphatidylinositol (GPI)-anchored proteins in epithelial cells. J. Cell Biol. **140:** 617–626
105 Nelson W. J. and Yeaman C. (2001) Protein trafficking in the exocytic pathway of polarized epithelial cells. Trends Cell Biol. **11:** 483–486
106 Gan Y., McGraw T. E. and Rodriguez-Boulan E. (2002) The epithelial-specific adaptor AP1B mediates post-endocytic recycling to the basolateral membrane. Nat. Cell Biol. **4:** 605–609
107 Simons K. and Toomre D. (2000) Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. **1:** 31–39
108 Sarnataro D., Nitsch L., Hunziker W. and Zurzolo C. (2000) Detergent insoluble microdomains are not involved in transcytosis of polymeric Ig receptor in FRT and MDCK cells. Traffic **1:** 794–802
109 Polishchuk R. S. and Lippincott-Schwartz J. (2001) In vivo dynamics of apical and basolateral cargo proteins between Golgi and plasma membrane. Mol. Biol. Cell. **12:** 338a
110 Yeaman C., Grindstaff K. K. and Nelson W. J. (1999) New perspectives on mechanisms involved in generating epithelial cell polarity. Physiol. Rev. **79:** 73–98
111 Morin-Ganet M. N., Rambourg A., Deitz S. B., Franzusoff A. and Kepes F. (2000) Morphogenesis and dynamics of the yeast Golgi apparatus. Traffic **1:** 56–68
112 Reiner D. S., McCaffery M. and Gillin F. D. (1990) Sorting of cyst wall proteins to a regulated secretory pathway during differentiation of the primitive eukaryote, *Giardia lamblia*. Eur. J. Cell Biol. **53:** 142–153
113 Taylor R. S., Jones S. M., Dahl R. H., Nordeen M. H. and Howell K. E. (1997) Characterization of the Golgi complex cleared of proteins in transit and examination of calcium uptake activities. Mol. Biol. Cell **8:** 1911–1931
114 Aridor M., Bannykh S. I., Rowe T. and Balch W. E. (1999) Cargo can modulate COPII vesicle formation from the endoplasmic reticulum. J. Biol. Chem. **274:** 4389–4399
115 Rambourg A., Clermont Y., Chretien M. and Olivier L. (1993) Modulation of the Golgi apparatus in stimulated and non-stimulated prolactin cells of female rats. Anat. Rec. **235:** 353–362