The role of the cytoskeleton in the structure and function of the Golgi apparatus

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Introduction

Cellular organelles in mammalian cells are individualized membrane entities that often become spherical. The endoplasmic reticulum (ER) and the Golgi apparatus (GA) are exceptions to this rule, as they are respectively made up of a continuous tubular network and a pile of flat disks. Their unique shapes are regulated by molecular elements (Kepes et al. 2005; Levine and Rabouille 2005), including the cytoskeleton. All cytoskeletal elements, together with cytoskeleton-associated motors and non-motor proteins, have a role in the subcellular positioning, biogenesis and function of most organelles, being particularly relevant in the GA.

The GA is the central organelle of the eukaryotic secretory pathway. While its basic function is highly conserved, the GA varies greatly in shape and number from one organism to another. In the simplest organisms like budding yeast *Saccharomyces cerevisiae*, the organelle takes the form of dispersed cisternae or isolated tubular networks (Preuss et al. 1992; Rambourg et al. 2001). Unicellular green alga (Henderson et al. 2007) and many protozoa like *Toxoplasma gondii* (Pelletier et al. 2002) and *Trypanosoma brucei* (He 2007; He et al. 2004) contain a single pile of flattened cisternae aligned in parallel. The organization of the GA in this manner is referred to as a Golgi stack, which usually contains two regions: one central and poorly fenestrated (compact) and other lateral and highly fenestrated (non-compact) (Kepes et al. 2005) (see the 3D modelling of a GA stack of a control NRK cell in Fig. 2). In fungi (Mogelsvang et al. 2003; Rossanese et al. 1999), plants (daSilva et al. 2004; Hawes and Satiat-Jeunemaitre 2005) or *Drosophila* (Kondylis and Rabouille 2003) many separate Golgi stacks are dispersed throughout the cytoplasm. In all these cases, each Golgi stack is associated with a single ER exit site (ERES), forming a secretory unit. In contrast, in most mammalian cells, the GA is a single-copy organelle shaped like a ribbon, containing numerous stacks joined by a tubular network and located near the nucleus (Ladinsky et al. 1999; Rambourg and Clermont 1986). In mammals, Golgi stacks are segregated from the ERES, and the Golgi ribbon is closely associated with the centrosome, the main organizing centre for cytoplasmic microtubules (MTOC) (Rios and Bornens 2003; Saraste and Goud 2007).

The cytoskeleton imposes the localization of the GA. Depending on the cellular model, either microtubules (MTs) or actin filaments (AFs) have the

greater influence (for instance, in mammalian and plant cells, respectively). Historically, the first cytoskeleton element to be linked to the GA and associated membrane trafficking was the MT (Thyberg and Moskalewski 1999). Some time later, both AFs and actin-associated proteins were clearly implicated (for recent reviews see Egea et al. (2006); Lanzetti (2007); Ridley (2006); Smythe and Ayscough (2006); Soldati and Schliwa (2006)), and more recently intermediate filaments (IFs) also appear to interact with Golgi membranes and participate in protein trafficking (Gao and Sztul 2001; Styers et al. 2005; Toivola et al. 2005). In this chapter, we provide a general overview of the structural and functional consequences of the coupling between the cytoskeleton and the GA in various cell models.

Microtubules and the structure and dynamics of the Golgi apparatus

Microtubules and the structural integrity of the Golgi apparatus

In non-polarized mammalian cells, the GA is closely associated with the centrosome, which is usually located near the nucleus at the cell centre (Rios and Bornens 2003) (Fig. 1). The spatial proximity of the GA and the centrosome has been known since Camilo Golgi's time, but it has been confirmed by immunofluorescence studies in the last 20 years. The close association with the centrosome is maintained even under conditions where cellular architecture is undergoing major remodelling, which occurs during cell migration (Kupfer et al. 1982), fusion of myoblasts to form myotubes (Ralston 1993; Tassin et al. 1985b), the delivery of lytic granules at the immunological synapse (Stinchcombe et al. 2006), phagocytosis (Eng et al. 2007; Stinchcombe et al. 2006) or neuronal polarization (de Anda et al. 2005). However, this association is broken when MT dynamics is perturbed by drugs like nocodazole (NZ) or taxol (TX) (Sandoval et al. 1984; Wehland et al. 1983), which suggests that the main factor governing Golgi ribbon integrity and localization is the microtubular network (Thyberg and Moskalewski 1985, 1999).

Pioneer studies of the role of MTs in the structural organization of the mammalian GA used drugs that favour MT disassembly (Robbins and Gonatas 1964). In the absence of MTs, the Golgi ribbon fragments, giving rise to discrete Golgi elements or mini-stacks, which are dispersed throughout the cell (Fig. 1). However, the GA does not need to be either intact or near the nucleus for protein transport or glycosylation (Rogalski et al. 1984). NZ-induced Golgi mini-stacks localize at the peripheral ERES, thus enabling the cell to maintain secretory transport from the ER (Cole et al. 1996; Trucco et al. 2004). Therefore, the GA of mammalian cells lacking MTs resembles the normal state of affairs in plant cells and fungi, where Golgi architecture and function occur without centralization.

Recent developments in microscope technology have advanced our understanding of the dynamics of MTs and GA interaction. For example, 3D electron microscope studies have allowed individual MTs to be modelled

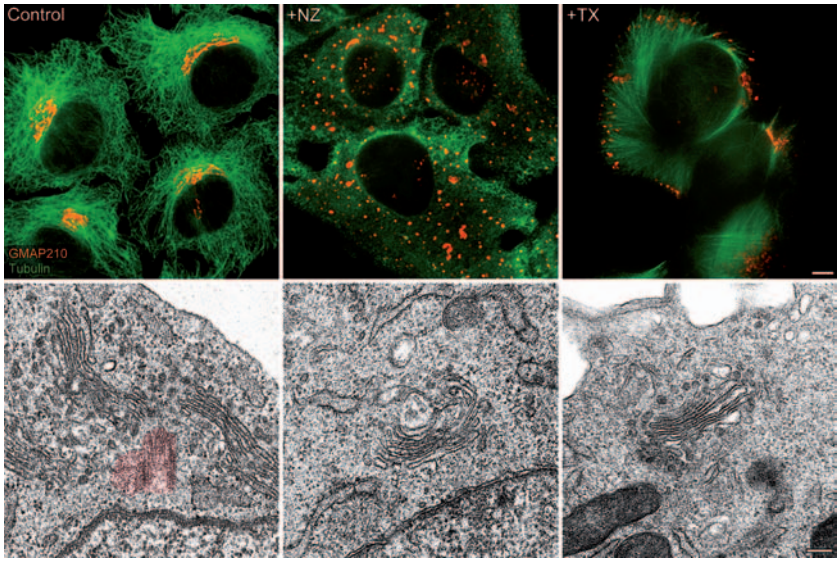

Figure 1. Microtubule–Golgi interaction. Immunofluorescence images of human retinal pigment epithelial cells fixed with methanol and double labelled for tubulin (green) and the Golgi protein GMAP210 (red). Cells were treated either with nocodazole (+NZ; 10 μM/3 h) to depolymerize MTs or with taxol (+TX; 10 μM/5 h) to induce the complete polymerization and stabilization of tubulin into MT bundles. Control, NZ- and TX-treated cells were also processed for electron microscopy analysis. Control cells show the characteristic ribbon-like arrangement of numerous adjacent Golgi stacks localised around centrioles (coloured in red). After NZ or TX treatments, numerous discrete Golgi elements appeared which maintained the characteristic stacked morphology (mini-stacks). Note that no significant differences in the ultrastructure of mini-stacks are seen between the two treatments. In TX-treated cells, Golgi mini-stacks are mostly localised to the cell periphery, whereas those in NZ-treated cells are uniformly distributed throughout the cytoplasm. Bars: 5 μm (immunofluorescence microscopy images) and 200 nm (electron microscopy images).

and the relationships with cisternae to be analyzed in situ. MTs specifically associate with the first *cis*-cisterna over long distances. MTs also cross Golgi stacks at multiple points via non-compact regions and cisternal openings (Marsh et al. 2001). Time-lapse microscopy studies have revealed that the overall 3D arrangement of the GA near the centrosome is relatively stable (Presley et al. 1997; Scales et al. 1997; Sciaky et al. 1997) although thin tubules are constantly formed and detached from the lateral portions of the GA. After extending from the GA, the tubules break off and move along MTs to the cell periphery. Some move directly to the ERES and, once attached, they collapse into them delivering Golgi proteins to the ER (Mardones et al. 2006). The motion of membrane elements from the ER to the GA is also critically dependent on MTs. If MTs are depolymerized, Golgi proteins that have recycled back to the ER are exported into pre-Golgi intermediates, which

then fail to move to the pericentrosomal region and consequently, remain stationary in the vicinity of ERES. Over long periods of time, these *de novo* structures acquire a normal Golgi stack morphology and become completely functional for secretion (Trucco et al. 2004). These studies indicate that the ability to form a Golgi stack is an intrinsic property of ER-derived membranes that does not require MTs. However, they are required to link stacks into a single organelle and to ensure its central location around the centrosome.

Another interesting aspect is the relationship between the GA and stable MTs. These MTs are characterized by the presence of detyrosinated and/or acetylated tubulin. They have a longer half-life and are more resistant to NZ-induced depolymerization (Schulze et al. 1987). Most stable MTs, which often appear short and convoluted under the fluorescence microscope, concentrate around the centrosome and colocalize with the GA (Burgess et al. 1991; Skoufias et al. 1990; Thyberg and Moskalewski 1993). Immunoelectron microscopy further demonstrated a close connection between detyrosinated MTs and vesicles transporting newly synthesized proteins from the ER to the Golgi (Mizuno and Singer 1994). Therefore, it has been proposed that there is a reciprocal relationship between MT stabilization and Golgi membrane dynamics. More than 10 years later, the molecular mechanisms mediating this relationship are now beginning to be unveiled (see below).

Microtubule-motor proteins

In the current view, it is difficult to understand how MTs contribute to the Golgi structure without considering how MTs mediate in Golgi-associated transport functions. In non-polarized cells, MTs are organized in a characteristic radial pattern with minus-ends anchored at the centrosome and plus-ends extending toward the cell periphery. Since the GA localizes around the centrosome, the predominant-associated motor activity involved should be minus-end directed. In eukaryotic cells, the primary molecular motor for minus-end-directed movements is cytoplasmic dynein 1 (Hook and Vallee 2006). Movement of transport carriers from peripheral ERES to the GA in the cell centre along MTs is mediated by dynein (Corthesy-Theulaz et al. 1992; Harada et al. 1998). In contrast, movement of membranes from the GA back to the ER is plus-end-directed and mediated by kinesin-2 (Stauber et al. 2006). Kinesins are a large protein superfamily, most of whose members have plus-end-directed activity (Miki et al. 2005). Dynein and kinesin are structurally similar, consisting of two functional parts: a motor domain that reversibly binds to the cytoskeleton and converts chemical energy into motion, and a tail that interacts with cargo either directly or through accessory chains (Caviston and Holzbaur 2006).

The mechanisms by which motors interact with a diversity of cargoes and subcellular targeting sites are not completely understood. One candidate factor proposed to link dynein to endomembranes is the multiprotein complex dynactin, an essential activator for most cytoplasmic dynein functions. Dynactin contains 11 subunits organized into an elaborate structure. The best

characterized subunits are the Arp1 filament, $p150^{Glued}$ and p50 dynamitin. $p150^{Glued}$ is a dimer that forms a coiled-coil and binds to dynein, the ARP1 filament and MTs. Dynamitin is required for maintaining the integrity of the complex (Schroer 2004). Expression of a dominant negative form of $p150^{Glued}$ or overexpression of dynamitin induces the fragmentation of the GA into multiple dispersed elements (Burkhardt et al. 1997; Quintyne et al. 1999). The movement of transport carriers from the ER to the GA along MT tracks is blocked under these conditions, as occurs in NZ-treated cells (Presley et al. 1997). Most likely, this blockade occurs at the earliest phases of ER protein export, since the Sec23p component the COPII complex interacts directly with dynactin. This interaction would facilitate the formation of transport carriers and their motion to the GA (Watson et al. 2005). It is therefore widely accepted that the dynein/dynactin motor is primarily responsible for ER-to-Golgi transport and the localization of the GA in the cell centre. An alternative model postulates that dynactin plays a role in coordinating the activity of opposing MT-motors and in regulating their processivity (Berezuk and Schroer 2007; Deacon et al. 2003; Haghnia et al. 2007; Ross et al. 2006). Supporting this view, dynein and kinesin colocalize in the same membrane structures (Welte 2004). Even more relevant for Golgi dynamics, kinesin-2 interacts with dynactin (Deacon et al. 2003). This interaction appears to involve primarily the non-motor subunit of kinesin-2 (KAP3) and the $p150^{Glued}$ subunit of dynactin. Knockdown of KAP3 blocked the Golgi-to-ER pathway and disorganized Golgi membranes (Stauber et al. 2006). The observation that kinesin-2 binds the same dynactin subunit as dynein raises the possibility that it could act as a molecular switch that coordinates bidirectional trafficking.

Recent data suggest that the scenario could actually be more complicated. Thus, dynein associates with Golgi membranes through several distinct mechanisms and dynactin, via $p150^{Glued}$, also binds Golgi-associated non-motor proteins. Dynein intermediate chain directly interacts with huntingtin, which has an important role in vesicle transport. Huntingtin silencing disrupts the GA in HeLa cells (Caviston et al. 2007). ZW10, a mitotic checkpoint protein that anchors dynein to kinetochores, also performs important functions in membrane traffic. These include dynein targeting to the GA and other membranes, but also SNARE-mediated ER–Golgi trafficking. ZW10 depletion provokes Golgi dispersal and decreases the frequency of minus-end-directed movements (Arasaki et al. 2006; Hirose et al. 2004; Vallee et al. 2006; Varma et al. 2006).

Interestingly, the small GTPases Cdc42 and Rab6 also play a role in regulating motor recruitment to membranes (Chen et al. 2005a; Matanis et al. 2002). Coatomer-bound Cdc42 prevents dynein binding to COPI vesicles, and expression of constitutively active Cdc42 blocks translocation toward the cell centre of NZ-induced stacks and ER-to-Golgi carriers (Chen et al. 2005a). Rab6 family members are involved in some MT-dependent transport steps from the *trans*-Golgi network (TGN). When Rab6 is activated, BicaudalD1/2 is recruited

to the TGN, which in turn recruits dynein–dynactin complexes (Hoogenraad et al. 2001; Matanis et al. 2002). It has been proposed that these complexes could participate in a recycling pathway that begins at the TGN and leads directly to the ER (Young et al. 2005). However, a later study showed that the major target for the fusion of Rab6-containing vesicles is the plasma membrane and that Rab6 regulates the transport and targeting of constitutive secretion vesicles. This study also reports an additional interaction of BicaudalD1/2 with kinesin-1. Therefore, the interactions between Rab6, BicaudalD1/2, kinesin-1 and dynein–dynactin complexes may contribute to the regulation of motor recruitment and co-ordination of their activities to specify directionality (Grigoriev et al. 2007; Saraste and Goud 2007). Defining the relative contributions of all of these mechanisms to the overall regulation of Golgi dynamics will require further investigation.

Role of the Golgi apparatus in microtubule dynamics

A new concept is emerging concerning the role of the GA in MT dynamics: the GA acting as a secondary MTOC (Luders and Stearns 2007). The ability of Golgi membranes to assemble and stabilize MTs was first noticed in hepatic cells after NZ treatment (Chabin-Brion et al. 2001). During NZ recovery, short MTs were invariably seen to associate with Golgi mini-stacks. In addition, purified Golgi membranes were shown to contain α-, β- and γ-tubulin and to support MT nucleation. However, this study did not resolve whether the MT nucleation was primarily carried out by the centrosome (the MTs then being released and anchored to Golgi membranes) or directly by the GA. Experimental support for the latter hypothesis came from the analysis of the mechanisms regulating the centering of a radial array of MTs in cells lacking centrosomes (Malikov et al. 2005). In stimulated cytoplasmic fragments of melanophores, pigment granules form a central aggregate that becomes the focal point from which MTs radiate. Radial MT arrays also form and become centralized in centrosome-free cytoplasts obtained from non-pigment cells. Strikingly, the GA appeared to be located in the centre of the cytoplasts, close to the MT aster (Malikov et al. 2004). Recently, the GA has been unambiguously identified as an MTOC by laser ablation of the centrosome (Efimov et al. 2007). MT-nucleation at the GA was shown to require γ-tubulin complexes. To date, two γ-tubulin-interacting proteins have been associated with the GA: GMAP210 and AKAP450/CG-NAP. The former is a *cis*-Golgi-associated protein that copurifies with MTs (Infante et al. 1999; Kim et al. 2007) and recruits γ-tubulin complexes to the GA (Rios et al. 2004). GMAP210 depletion fragments the Golgi ribbon into elements that remain near the centrosome (Rios et al. 2004). AKAP450 localizes at both the centrosome and the GA (Keryer et al. 2003a, b; Larocca et al. 2004; Takahashi et al. 1999). It contains two MT-binding domains and interacts with γ-tubulin complexes, thus providing MT-nucleating sites to the centrosome and, probably, to the GA as well (Kim et al. 2007; Takahashi et al. 2002). Interestingly, AKAP450 also interacts with $p150^{Glued}$ and the expression of a mutant that disrupts this interaction causes

GA fragmentation and dispersion in a similar manner to that observed with the overexpression of dynamitin.

In RPE-1 cells, many MTs are generated from the TGN, where microtubule plus-end-binding proteins CLASPs localize. These proteins stabilize pre-existing MT seeds by coating them, thus preventing their disassembly. In this regard, the centrosomal protein CAP350, which was originally believed to participate in MT-anchoring at the centrosome (Yan et al. 2006) actually stabilizes MTs enriched in the Golgi complex, and thus helps to maintain the integrity of the GA in the vicinity of the centrosome (Hoppeler-Lebel et al. 2007).

Relationship between the Golgi apparatus and microtubules in different cellular systems

So far, we have focused on what we have learned in cells displaying a radial MT array with plus-ends facing toward the cell cortex and minus-ends anchored at the centrosome. Direct observation of MTs reveals that cell lines with well-defined radial MT arrays are really a minority. In contrast, most cell lines display a loosely organized MT array. These differences are due to variations in the number of centrosomal anchored MTs, which ranges from their totality (lymphocytes) to practically none (epithelia) (Bornens 2002). In parallel, the morphology of the GA varies from a fully compacted shape around the centrosome to a highly extended one (Rios and Bornens 2003). The differentiation of specialized cells types in multicellular organisms frequently leads to the generation of non-radial MT arrays, which better serve the specialized functions of these cells (Dammermann et al. 2003; Musch 2004). Two of the most representative examples of non-radial MT arrays are the polarized epithelial cell and skeletal muscle fiber.

In polarized epithelial cells, MTs form an apico-basal array with their minus-ends concentrated near the apical surface and their plus-ends facing the basal domain (Mogensen et al. 2000). This array determines the membrane trafficking, which is central to the function of epithelia. In addition to vertically arranged MTs, cell lines derived from columnar epithelia (MDCK or Caco-2) also show networks of horizontal MTs both at the cell apex and the cell base. In hepatocytes, MTs converge underneath the bile-canalicular lumen at the apical surface (Dammermann et al. 2003; Musch 2004). The GA shows compact morphology and typically lies just apical to the nucleus, well-separated from the microtubule minus-ends. In MDCK cells, the GA extends upwards from the nucleus to the apical portion of the cell. At the same time, in epithelial cells, membrane proteins are segregated into functionally and structurally different apical and basolateral domains. Whereas MTs seem to be important in the organization of apical exocytosis, the actin cytoskeleton seems to be the main organizer for basolateral secretion. However, neither the motors that participate in the exit of various classes of proteins from the TGN, nor the molecular interactions that allow MTs and actin filaments to modulate luminal and basolateral polarity are fully understood (Rodriguez-Boulan et al. 2005).

Skeletal muscle fibers are multinucleate cells resulting from the fusion of mononucleate myoblasts in myogenesis. During this process, both pericentriolar proteins and MT nucleation sites redistribute from the centrosome to the nuclear periphery (Dammermann et al. 2003; Tassin et al. 1985a). In the same way, the GA is redistributed into smaller perinuclear elements that are formed by stacked cisternae (Tassin et al. 1985b). As in non-polarized cells, these perinuclear Golgi elements appear localized near the ERES and associated with stable MTs (Lu et al. 2001; Percival and Froehner 2007; Ralston et al. 1999, 2001).

The actin-based cytoskeleton and the Golgi apparatus

The actin-based cytoskeleton and the structural organization of the Golgi apparatus in mammalian cells

The first experimental evidence that AFs and the GA are linked was the observation that the GA invariably becomes compacted when a large variety of clonal cell lines are treated with actin toxins that either depolymerize (mainly cytochalasins and latrunculins) or stabilize and nucleate AFs (jasplakinolide) (di Campli et al. 1999; Lazaro-Dieguez et al. 2006; Valderrama et al. 1998, 2000, 2001) (Fig. 2). However, at the ultrastructural level, Golgi stacks from cells treated with actin-depolymerizing or -stabilizing toxins appeared different. Thus, the former mainly show swelled cisternae, while the latter have perforated/fragmented cisternae, which remain totally flat (Fig. 2). Supporting these observations, cisternae in NZ-treated cells remain completely flat (Thyberg and Moskalewski 1999; Trucco et al. 2004), and the GA in cells treated with NZ plus actin toxins display the same ultrastructural alterations as those seen in cells treated with actin toxins alone (Lazaro-Dieguez et al. 2006). This indicates that there is no synergic cooperation between MTs and AFs controlling the cisterna morphology. Therefore, as a general rule, MTs determine the pericentriolar localization of the Golgi ribbon, whereas AFs maintain the shape and membrane integrity of cisternae.

Cisternae are always flat, despite the huge amount of cargo that is continuously crossing the Golgi stack. When the amount of cargo to be transported is much higher, then membrane continuities appear between cisternae (Trucco et al. 2004). The aforementioned ultrastructural changes induced by actin toxins indicate that AFs provide the necessary mechanical stability to cisternae to prevent their expected spontaneous swelling as a consequence of the hyperosmotic protein content in transit through the Golgi stack. By analogy with red blood cells, the unique flat morphology of cisternae could result from the structural organization of the spectrin-actin-based cytoskeleton present in the GA (Bennett and Baines 2001; De Matteis and Morrow 2000). In this respect, spectrin and ankyrin isoforms, actin, and an anion exchanger (AE2) are all present in Golgi membranes (Beck et al. 1997; Devarajan et al. 1996, 1997; Godi et al. 1998; Heimann et al. 1999; Holappa et al. 2001, 2004; Stankewich et al. 1998; Valderrama et al. 2000). Together

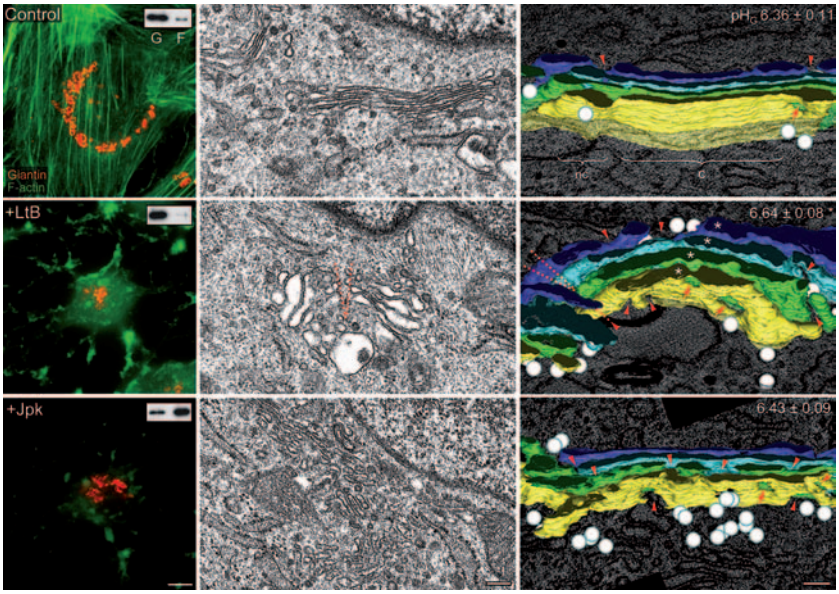

Figure 2. Actin filaments–Golgi interaction. NRK cells treated with the actin-depolymerizing toxin latrunculin B (+LtB; 500 nM/45 min) or the actin-stabilizing toxin jasplakinolide (+Jpk; 500 nM/45 min) show a similar compaction of the Golgi complex when viewed under the fluorescence microscope. In the respective panels, we also show the resulting F- and G-actin pools. At ultrastructural level, LtB treatment mainly produced swelling of cisternae. In contrast, Jpk treatment only induced fragmentation of cisternae. We also display 3D reconstructions obtained from electron tomograms of the GA from control and actin toxin-treated cells. In control cells, both the central compact (c) and the lateral non-compact (nc) regions are seen. In LtB-treated cells, the predominant alteration is the swelling of cisternae (asterisks), but some cisternal perforation/fragmentation is also seen (arrowheads). Jpk treatment left cisternae flat but they show numerous perforations (arrows) and perforations/fragmentations (arrowheads). A perforation/fragmentation of a Golgi stack is indicated by the dashed red lines. We also indicate, in the respective 3D Golgi modelling panels, the intra-Golgi pH values obtained for each experimental condition (asterisk indicates a statistical significance of $p \leq 0.01$ according to the Student's *t*-test).

with these, ion regulatory molecules such as vacuolar H^+-ATPases (Moriyama and Nelson 1989) and cation (NHEs) exchangers (Nakamura et al. 2005) resident in the Golgi or in transit to the plasma membrane could contribute to this postulated actin/spectrin-based cisternal mechanical stability by providing the appropriate intra-Golgi ion and pH homeostasis. This is essential, on the one hand for Golgi-associated post-translational protein and lipid modifications (Axelsson et al. 2001) and on the other hand to keep the cisternae flat. Thus, bafilomycin A1, an inhibitor of vacuolar ATPases, both induces cisterna swelling and slows the Golgi-to-ER protein transport (Palokangas et al. 1998). Curiously, the cisterna swelling after AFs depolymerization is accompanied by an increase in the intra-Golgi pH (Fig. 2). The restitution of normal actin

cytoskeleton organization after the removal of actin-depolymerizing toxins is followed by the normalization of cisterna morphology and the intra-Golgi pH (Lazaro-Dieguez et al. 2006). This correlation strongly suggests that AFs could interact and modulate the activity of (some) ionic regulatory proteins (vacuolar ATPases, anion and cation exchangers, ionic channels, pumps, and/or transporters) present in Golgi membranes. This interaction would be highly similar to that observed for some of these proteins present at the plasma membrane. Therefore, we postulate that the equilibrium of osmotically active ions would maintain the flatness of Golgi cisternae in concert with the actin assembly state (Lazaro-Dieguez et al. 2006). At the same time, the Golgi-associated spectrin-actin cytoskeleton system could organize the secretory molecular machinery controlling the lateral distribution of the main Golgi membrane components (De Matteis and Morrow 2000). Thus, a physical barrier could be formed in the compact region of cisternae by the conjunction of the spectrin-actin-based cytoskeleton together with particular lipids (for example, cylindrical-shaped ones) and proteins (for example, glycosyltransferases). This would result in a permanent inhibitory membrane area for the biogenesis of transport carriers. Future research in the Golgi spectrin and ankyrin isoforms should provide significant insights into Golgi architecture.

The actin-based cytoskeleton and the biogenesis and motion of Golgi-derived transport carriers

The presence in Golgi membranes of molecular components that trigger actin polymerization (Arp2/3, Cdc42, cortactin, N-WASP, syndapin) and those that determine vesicular budding (coatomer and clathrin coats) and fission (dynamin) suggests their physiological coupling, as occurs at the plasma membrane during endocytosis. Actin assembly provides the necessary structural support that facilitates the formation of transport carriers in the lateral portions of Golgi membranes. This can be achieved by generating force through de novo actin polymerization, which in turn can be accompanied by the mechanical activity of actin motors (myosins). In respect to the former possibility, the actin nucleators Arp2/3 and Spir1 are present in the Golgi (Carreno et al. 2004; Chen et al. 2004; Kerkhoff et al. 2001; Matas et al. 2004). Their respective upstream regulators can be diverse. For the Arp2/3 complex, the more consistent are Cdc42-N-WASP and dynamin2-cortactin (Cao et al. 2005; Chen et al. 2004; Luna et al. 2002; Matas et al. 2004). At the *trans*-Golgi network (TGN), there is experimental evidence of the functional coupling between dynamin-mediated membrane fission and Arp2/3-mediated actin-based mechanisms (Cao et al. 2005; Carreno et al. 2004; Kerkhoff et al. 2001; Kessels and Qualmann 2004; Praefcke and McMahon 2004; Rozelle et al. 2000). Thus, the interference with dynamin2/cortactin or syndapin2/dynamin2 protein interactions blocks post-Golgi protein transport (Cao et al. 2005; Kessels et al. 2006). Fewer data are available on early Golgi compartments, but an interesting functional connection between actin polymerization governed by Cdc42, coatomer (COPI)-mediated transport carrier formation,

and microtubule motor-mediated motion has been described (Chen et al. 2005a). Under the activation of the ADP-ribosylation factor 1 (ARF1), actin, coatomer and the Cdc42 are all recruited to Golgi membranes (Stamnes 2002). Cdc42 interacts with γ-COP subunit of the COPI-coated transport carrier in a cargo receptor p23-sensitive manner such that coatomer cannot simultaneously bind to Cdc42 and p23 (Chen et al. 2005a, b). Interestingly, the activation of Cdc42 (Cdc42-GTP) inhibits the recruitment of dynein to COPI-coated transport carriers. In contrast, the prevention of the COPI–Cdc42 interaction by p23 stimulates dynein recruitment on Golgi-derived transport carriers, and hence their MT-based transport. Overall, this could provide a safe control mechanism by which a COPI-mediated transport carrier cannot be moved (through microtubule motors) until it is completely assembled (when Cdc42 does not bind to coatomer) (Hehnly and Stamnes 2007). Supporting this idea, the disruption of actin filaments as well as the activation of the Cdc42-N-WASP-Arp2/3 signaling pathway by the expression of the constitutively activated mutant of Cdc42 (GTP-bound) block the COPI-mediated Golgi-to-ER protein transport (Luna et al. 2002; Valderrama et al. 2001). Therefore, the local fine regulation of the actin dynamics state on the transport carrier assembly could represent an early step that precedes the scission of the transport carrier in the lateral portions of cisternae for its subsequent switching to MT tracks and motility (Egea et al. 2006).

The coupling between actin polymerization and transport carrier biogenesis occurs both at the TGN and in early Golgi compartments, but some of the molecular mediators that regulate both processes are unevenly distributed in the GA. Thus, regarding actin polymerization, Cdc42 and Arp2/3 are present to varying degrees in the Golgi stack (*cis*/middle/*trans*-cisternae) and at the TGN, but N-WASP is absent from the *trans*/TGN (Matas et al. 2004). Cortactin, which like N-WASP also recruits Arp2/3, is visualized at the tips and buds at both the *cis*- and *trans*-cisternae (Cao et al. 2005). It is then reasonable to postulate that, at the TGN, cortactin substitutes N-WASP in the recruitment of the Arp2/3 that mediate in the post-Golgi protein transport. If so, the *cis*-to-*trans*/TGN segregation of some fundamental components involved in actin polymerization would facilitate the targeting and assembly of the specific molecular machinery that participates in the membrane budding and fission occurring in Golgi compartments. Thus, the sequential protein interactions syndapin2- or dynamin2-cortactin-Arp2/3 and Cdc42-N-WASP-Arp2/3 are respectively restricted to the TGN and to early Golgi compartments, participating in this manner in the post-Golgi and in the ER/Golgi interface protein transport.

A key aspect in the structure of polarized cells (epithelial and neuronal) is the maintenance of polarized organization based on highly specific sorting machinery for cargo destined to the apical or basolateral membrane domain at the exit of the TGN (Rodriguez-Boulan et al. 2005). In accordance with the localization of Cdc42 in the *trans*/TGN (Matas et al. 2004), the expression of constitutively active (GTP-bound) or inactive (GDP-bound) Cdc42 mutants

slows the exit of basolateral protein markers and accelerates apical ones (Cohen et al. 2001; Kroschewski et al. 1999; Musch et al. 2001). The downstream effectors involved in the regulatory protein sorting induced by Cdc42 at the TGN are unknown. The integrity of AFs is necessary for efficient delivery of some (but not all) proteins to the apical domain. For example, in MDCK cells the apical delivery of sucrase-isomaltase, but not that of lactase-phlorizin hydrolase or gp80, occurs along AF tracks (Delacour and Jacob 2006; Jacob et al. 2003). Interference with actin dynamics using actin toxins variably affects the exit of apical- and basolateral-targeted cargo from the TGN. Moreover, actin does not participate in the TGN egress of lipid raft-associated GPI-anchored cargo (Lazaro-Dieguez et al. 2007).

Actin nucleation/polymerization activity on Golgi membranes can also give rise to the formation of actin comet tails, which consist of filamentous actin and various actin-binding proteins that focally assemble and grow on a membrane surface (Welch and Mullins 2002). Actin tails have been observed in raft-enriched TGN-derived vesicles in certain experimental conditions (Rozelle et al. 2000), but this does not seem to be the most efficient mechanism to specify directionality to transport carriers. In this respect, MT and AF tracks are more suitable. However, analogously to what happens at the plasma membrane (Merrifield 2004; Merrifield et al. 2005; Perrais and Merrifield 2005), Golgi-associated Arp2/3-mediated actin polymerization generates a force. Depending on whether this acts on the lateral portion of the cisterna or on the transport carrier membrane, it could respectively facilitate the membrane elongation that precedes membrane scission or propel the transport carrier away from the cisterna. The presence of components of the Arp2/3 complex in cisternae and periGolgi transport carriers (Chen et al. 2005a; Matas et al. 2004) endorses both possibilities, but direct experimental evidence of either phenomenon is still lacking. However, an interesting in vitro approach has recently been reported (Heuvingh et al. 2007). These authors observed actin polymerization around liposomes composed of a specific lipid that facilitates the recruitment of the activated form of ARF1. This actin polymerization was dependent on Cdc42 and N-WASP present in HeLa cell extracts, and resulted in the formation of actin comets, which pushed the ARF1 liposomes forward.

Tight control of the coupling between Golgi-associated actin polymerization and membrane elongation and fission reactions prevents the structural and functional collapse of the GA. Part of this control can be achieved by regulating the activation state of Cdc42 in Golgi membranes. The Cdc42 GAP (GTPase-activating protein) ARHGAP10 and GEFs (guanine nucleotide exchange factor) Fgd1 and Dbs are present in Golgi membranes (Dubois et al. 2005; Estrada et al. 2001; Kostenko et al. 2005). In addition, the low levels of phosphatidylinositol 4,5-biphosphate (PIP_2) present in the Golgi (De Matteis et al. 2005) could also facilitate this control. Note that PIP_2 synergizes with Cdc42-N-WASP and cortactin in Arp2/3-triggered actin nucleation/polymerization (Rohatgi et al. 2000; Schafer et al. 2002).

A variety of independent experimental approaches show that Cdc42 is the only Rho GTPase that functions in the Golgi complex in mammalian cell lines (Fucini et al. 2000; Matas et al. 2005; Prigozhina and Waterman-Storer 2004; Valderrama et al. 2000). However, neurons seem to be an exception. Citron-N, a RhoA-binding protein and ROCKinase-II are both seen in the neuronal GA (Camera et al. 2003). Likewise, LIMK1, a kinase that specifically phosphorylates ADF-cofilin, localizes to Golgi membranes (Rosso et al. 2004). For a review of the role of actin and actin-binding/regulatory proteins in the GA of neuronal cells see (14)■.

In addition to actin polymerization, myosins also generate a force, which can promote the formation of transport carriers and/or their movement away from Golgi membranes along AF tracks. Non-muscle myosin II mediates both Golgi-to-ER and post-Golgi protein transport (DePina et al. 2007; Duran et al. 2003; Musch et al. 1997; Stow et al. 1998). This myosin is a non-processive motor that directly interacts with Golgi membranes in vitro (Fath 2005). It is postulated that this motor is tethered to the cisterna by its tail and to actin filaments by its head. Its subsequent motion along actin filaments could provide the force needed to extend Golgi-derived membranes away from the cisterna. This would be similar to what happens to tubules originating from liposomes incubated with microtubular motors (kinesin) moving along microtubules (Roux et al. 2002). This cisterna-derived membrane extension could facilitate the subsequent functional coupling of membrane scission protein(s), leading to the complete release of the transport carrier. The binding of a tropomyosin isoform to Golgi-associated short actin filaments (Percival et al. 2004) could facilitate non-muscle myosin II recruitment and its interaction with them. Myosin VI is another myosin motor located in the GA (Buss et al. 2004; Warner et al. 2003). It differs from other processive myosins (for example, myosin V and X) as it only moves transport carriers towards the fast-depolymerizing minus-end pole of the microfilament. Therefore, myosin VI could provide the force and directionality for the transport carrier movement away from cisternae according to the expected fast-growing plus-end polarization of the actin filaments originated from Golgi membranes (Chen et al. 2004). The interaction between myosin VI and optineurin, a partner of Rab8 (Sahlender et al. 2005) extends to the Golgi–actin cytoskeleton interaction the known role of some Rab proteins as linkers of endomembrane systems to cytoskeletal motors (Jordens et al. 2005). Myosin VI, together with optineurin and Rab 8, operates in protein sorting and transport at the TGN in polarized cells (Au et al. 2007). The myosin VI–optineurin complex is required in the basolateral protein sorting pathway mediated by the Golgi-associated clathrin adaptor protein AP-1B, which in turn is specifically regulated by Rab 8 (Ang et al. 2003). The inhibition of myosin VI results in the incorporation of basolateral membrane proteins into apical transport carriers and their delivery to the apical plasma membrane domain. Sorting of other basolateral or apical cargo does not involve myosin VI. This result suggests that myosin motors could selectively couple protein sorting and transport carrier

biogenesis and motility. Finally, class I myosins are also reported to associate with Golgi membranes and on apical Golgi-derived vesicles from polarized cells (Fath and Burgess 1993; Jacob et al. 2003; Montes de Oca et al. 1997). In myosin Ia knock-out mice, apical markers sucrase–isomaltase and galectin-4 are mislocalized to the basolateral surface in intestinal epithelial cells (Tyska et al. 2005). The sorting ability of myosin I could be linked to its capacity to interact with lipid raft-associated cargo as this monomeric, non-processive motor binds to phospholipid vesicles (Hayden et al. 1990).

The Golgi apparatus–actin interaction in other cellular models

Yeast

The use of a large number of mutants that produce alterations in intracellular traffic in the budding yeast *Saccharomyces cerevisie* has led to the identification of proteins involved in both membrane trafficking and actin organization (Kaksonen et al. 2006; Mulholland et al. 1997). Most components of the secretory pathway and many of the actin-based cytoskeleton are conserved between yeast and mammalian cells. The actin cytoskeleton in yeast consists primarily of cortical patches and cables (Adams and Pringle 1984; Kilmartin and Adams 1984; Moseley and Goode 2006). AFs, and not MTs, polarizes growth in yeast (Novick and Botstein 1985). In *Saccharomyces*, MTs have not been implicated in the dynamics of any organelle except the nucleus (Rossanese et al. 2001). Many actin mutants accumulate large secretory vesicles and exhibit phenotypes consistent with defects in polarized growth (Pruyne et al. 2004). This, together with the polarized organization of actin cytoskeleton, has suggested a role for actin in the polarized transport of late secretory vesicles to the plasma membrane (Finger and Novick 2000; Mulholland et al. 1997). Thus, a mutation of GRD20, a protein involved in sorting in the TGN/endosomal system, showed aberrant secretion of the vacuolar hydrolase carboxypeptidase Y, but not other TGN membrane proteins, as well as defects in the polarization of the actin cytoskeleton (Spelbrink and Nothwehr 1999). Recently, depletion of Av19p in a strain that also lacks Vps1 (dynamin) and Apl2 (adaptor–protein complex 1) proteins results in secretory defects, accumulation of Golgi-like membranes, and a non-polarized actin cytoskeleton organization (Harsay and Schekman 2007). Finally, concentration of late (but not early) Golgi elements in the sites of polarized growth (the bud) depends on actin, which is transported along actin cables by type V myosin Myo2p (Rossanese et al. 2001). With regard to the early secretory pathway, AFs depolymerization with actin toxins does not affect ER-to-Golgi (Brazer et al. 2000) or Golgi-to-ER (M. Muñiz, personal communication) protein transport. Taken together, these results demonstrate that in yeast, actin organization directly participates in post-Golgi vesicular transport and in the Golgi inheritance.

Drosophila

In adherent S2 cells derived from mixed *Drosophila melanogaster* embryonic tissues, it has recently been reported that Golgi inheritance occurs by

duplication to form a paired structure. This process requires an intact actin cytoskeleton and depends on Abi/Scar but not WASP (Kondylis et al. 2007). In another recent study, the analysis of a genome-wide RNA-mediated interference screen in these cells showed that the depletion of the *tsr* gene (which codifies for destrin, also known as ADF/cofilin) induces Golgi membranes to aggregate and swell, resulting in inhibition of the HRP secretion (Bard et al. 2006). Coronin proteins dpdo1 and coro regulate the actin cytoskeleton, but also govern biosynthetic and endocytic vesicular trafficking, as indicated by mutant phenotypes that show severe developmental defects ranging from abnormal cell division to aberrant formation of morphogen gradients (Rybakin and Clemen 2005).

Dictyostelium

The slime mould *Dictyostelium discoideum* (like *Drosophila*) is widely studied in developmental and cell biology. Cells of this protist are easy to manipulate by genetic and biochemical means. They contain various types of vacuole, ER and very small Golgi stacks (Becker and Melkonian 1996). Comitin (p24) is a dimeric *Dictyostelium* actin-binding protein present in the GA and in vesicle membranes that contains sequence motifs homologous to lectins. It is postulated that this protein may bind Golgi-derived vesicles to the actin filaments via the cytoplasmically exposed mannosylated glycans (Jung et al. 1996; Weiner et al. 1993). Villidin is another actin-binding protein present in this organism that seems to be associated with secretory vesicular and Golgi membranes (Gloss et al. 2003). LIS1 (DdLIS1) is a centrosomal protein required for the link between MTs, the nucleus and the centrosome that also controls the GA morphology. Mutants of this protein lead to MT disruption, Golgi fragmentation and actin depolymerization (Rehberg et al. 2005).

Caenorhabditis elegans

Very little is known about the GA and actin cytoskeleton interaction in this organism, but consistent with a possible role of coronin 7 in Golgi trafficking (Rybakin et al. 2004), depletion of POD-1 gene (a Coronin 7 homolog) using RNA interference leads to aberrant accumulation of vesicles in cells of the early embryo (Rappleye et al. 1999). Moreover, CRP-1, a Cdc42-related protein, localizes at the TGN and recycling endosomes. Alteration of CRP-1 expression in epithelial-like cells affected the apical but not the basolateral trafficking (Jenna et al. 2005).

Plant cells

The structural organization of the GA in plants has many points in common with animal cells but there are important differences, which are largely dependent on the different cytoskeleton organization of plant cells. Thus, interphase higher plant cells (angiosperms and some gymnosperms without flagellate sperm) lack doublet and triplet MTs and a single MTOC. Instead, numerous MTOCs are aligned in the cortex, which assemble and form the

transverse bands referred to as cortical MTs. These MTs are essential for the transport of Golgi-derived vesicles formed during metaphase (Segui-Simarro et al. 2004), which subsequently fuse to form the phragmoplast (Jurgens 2005), the equivalent to the contractile ring in animal cells. In contrast to MTs, stationary AFs are most prominent in plant cells (known as actin bundles) where they are all oriented with the same polarity and aligned along the plant cell. Attached to the actin bundles are the ER, vesicles and numerous discrete or a few clustered Golgi stack-TGN units (also named Golgi bodies or dictyosomes). Importantly, Golgi units are highly variable in number (a few tens to hundreds) depending on the plant type, cell type and the developmental stage of the cell (Hawes and Satiat-Jeunemaitre 2005; Kepes et al. 2005). In polarized root hairs and pollen tubes, the TGN is a vesicular-like non-tubular) compartment morphologically segregated from Golgi stacks. It localizes to growing tips of these cells, where together with actin, plant Rho/Rac members (ROPs and Rac1, respectively), Rab (Rab4a and Rab11) and ARF (ARF1) small GTPases regulate vesicular secretory and endocytic trafficking (Samaj et al. 2006).

In plants, most of the endomembrane compartments are in constant movement together with the cytoplasmic streaming whereby cellular metabolites are distributed all over the cell (Shimmen and Yokota 2004). ER vesicles and the Golgi units show actin-dependent dispersal and spatial organization and are propelled by the plant-specific myosin XI (Boutte et al. 2007). Discrete Golgi units contains a fine fibrillar material enriched in actin, spectrin and myosin-like proteins (especially the former) (Mollenhauer and Morre 1976; Satiat-Jeunemaitre et al. 1996). The depolymerization of AFs with actin toxins uncouples the association between specific regions of cortical ER with individual Golgi bodies (Boevink et al. 1998; Brandizzi et al. 2003). Thus, cytochalasin or latrunculin treatments induce the aggregation of Golgi bodies and variably alter the Golgi morphology. However, the latter depends on the cell type examined and the period of treatment (Chen et al. 2006; Satiat-Jeunemaitre et al. 1996). Actin toxins also perturb the coordinated movement of Golgi bodies and ER tubules (daSilva et al. 2004; Yang et al. 2005). Actin does not participate in the ER/Golgi interface protein transport (Saint-Jore et al. 2002), but it does it in post-Golgi trafficking to the plasma membrane and the vacuole. Thus, in the tip of growing cells like pollen tubes, AFs are the tracks on which Golgi-derived secretory vesicles are transported (Picton and Steer 1981; Vidali et al. 2001). Cargoes containing polysaccharides and the enzymes necessary for cell-wall morphogenesis also require an intact actin–myosin system (Blancaflor 2002; Hu et al. 2003; Miller et al. 1995; Nebenfuhr et al. 1999). Therefore, post-Golgi trafficking and the organization of vacuoles in plant cells require an intact actin cytoskeleton (Uemura et al. 2002).

The Golgi apparatus-intermediate filaments interaction

IFs are found in nearly all animal cells. They are classified according to their distribution in specific tissues. In contrast to MTs and AFs, IFs do not exhibit

polarity or bind nucleotides, and they are considered a more stable structure. IFs are of intermediate size (8–12 nm) in comparison to MTs (23–25 nm) and to AFs (6–8 nm). IFs maintain cell and tissue integrity thanks to their mechanical properties, cellular distribution and, as far as we know, from disease-associated IFs phenotypes. IFs participate in the regulation of key signaling pathways that control cell survival and growth, and also in protein targeting and membrane trafficking (Coulombe and Wong 2004; Kim and Coulombe 2007; Omary et al. 2004; Oriolo et al. 2007; Styers et al. 2005; Toivola et al. 2005). IFs extend from the plasma membrane to the nucleus in close vicinity to some organelles such as mitochondria, endocytic compartments, and the GA (Fig. 3).

The first Golgi–IF interaction was reported for vimentin filaments at ultrastructural level (Katsumoto et al. 1991), and later confirmed biochemically (Gao and Sztul 2001). The interaction is mediated by the Golgi

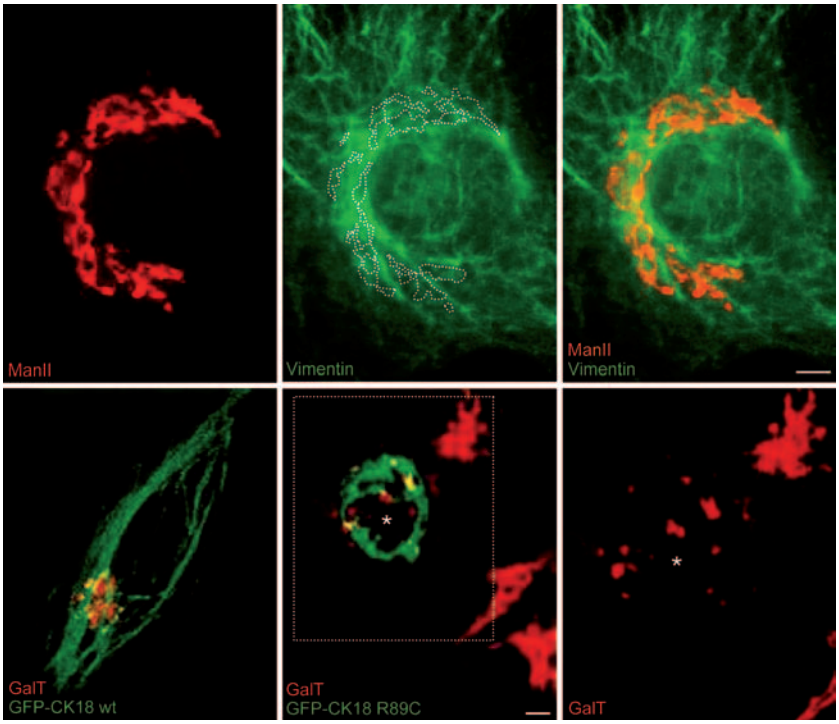

Figure 3. Intermediate filaments–Golgi interaction. The upper panels show an NRK cell double stained to reveal the association of the GA (using anti-mannosidase II antibodies; in red) with a network of vimentin intermediate filaments (in green) seen by immunofluorescence. Bar: 2 μm. The bottom panels illustrate the severe Golgi fragmentation that occurred in transfected Huh7 cells expressing the GFP-cytokeratin18 R89C mutant. The GA was revealed with anti-galactosyltransferase (GalT) antibodies. Neighbouring non-transfected cells show a normal GA (Kumemura et al. 2004) (images used with permission of the authors and the publisher). Bar, 4 μm.

membrane-associated protein formiminotransferase cyclodeaminase (FTCD), a metabolic enzyme involved in conversion of histidine to glutamic acid (Gao et al. 1998). Overexpression of FTCD resulted in the formation of extensive FTCD-containing fibres originating from the GA and inducing its fragmentation, and whose fragments remained tethered to these fibers (Gao and Sztul, 2001). However, the GA appears to be normal in vimentin–null cells (Gao et al. 2002; Styers et al. 2004). Thus, it is postulated that the vimentin–FTCD interaction at the GA is essential for FTCD functionality but not linked to the maintenance of Golgi organization. Oxysterol-binding protein (OSBP) regulates lipid and cholesterol metabolism and interacts with the GA in the presence of oxysterol (Ridgway et al. 1992). A splice variant form of the OSBP-related protein 4 in which the PH domain and part of the oxysterol-binding domain are deleted, colocalizes with vimentin IF in the Golgi region and inhibits the intracellular cholesterol transport pathway mediated by vimentin (Wang et al. 2002).

Epithelial cells express cytokeratins, whose mutations are also associated with epidermal, oral and ocular diseases (Uitto et al. 2007). Arginine 89 of cytokeratin18 plays an important role in IF assembly. The expression of this mutant cytokeratin-induced aggregations, loss of the cytokeratin cytoskeleton and the fragmentation of the GA (Fig. 3). Moreover, the Golgi reassembly occurring after NZ or brefeldin A treatments was perturbed only in cells expressing both cytokeratins and vimentin IFs (Huh7 and OUMS29 cells), but curiously not in cells that only possess vimentin IFs (HEK293 cells) (Kumemura et al. 2004).

Whether cytokeratin or vimentin IFs are involved in Golgi-associated protein sorting, vesicular formation and/or transport remains to be established. However, recent evidence indicates that IFs could regulate some membrane protein targeting events, which take place at the GA level. Thus vimentin directly binds AP-3 and thus regulates protein sorting in endo-lysosomes (Styers et al. 2004). Polarized enterocytes and hepatocytes depleted of some keratins by antisense strategies and in cytokeratin 8-null mouse cells showed altered apical protein transport (Ameen et al. 2001; Rodriguez et al. 1994; Salas et al. 1997). Maturation of glycosphingolipids is also impaired in vimentin-deficient cells, but the defect seems to be localized to the Golgi/endosomal interface transport (Gillard et al. 1994, 1998). Since MT-motors kinesin and dynein also control the dynamics of IFs (Helfand et al. 2002, 2003; Prahlad et al. 1998), the reported Golgi/endo-lysosomal membrane trafficking mediated by IFs may be under the control of the dynamic IF–MT interaction, which can also be applied to AFs and motors. In this respect, the actin-based motor myosin Va has been identified as a neurofilament-associated protein (Rao et al. 2002).

In summary, the structural and functional interaction of the GA with IFs is not yet firmly established. However, since IFs are well-integrated with both actin and microtubule cytoskeletons and their motors (Chang and Goldman 2004), the organization of the GA may also be influenced by the organization and dynamics of IFs.

Conclusion

Both MTs and AFs are necessary for correct Golgi positioning, architecture and trafficking. Strong evidence in favour of this view now indicates that the GA functions as a microtubule and as an actin-nucleating organelle. In general terms, the relationships between each cytoskeleton network and Golgi dynamics are complementary. Thus, in animals cells, the actin-dependent cytoskeleton (AFs and actin-binding/regulatory proteins) plays an important role in early events of vesicular transport (sorting and/or membrane fission), and in the maintenance of the flattened morphology of cisternae. Furthermore, MTs and associated motors are directly involved in the motion of Golgi-derived transport carriers to their final destinations and in the positioning and organization of the Golgi ribbon. In contrast, in plant cells, endomembrane organization and trafficking are almost exclusively mediated by AFs. Both ER and individual Golgi stacks are directly anchored to actin bundles, and transport between the ER and the GA is cytoskeleton-independent. In yeast, much less is known but both cytoskeleton elements participate in post-Golgi protein transport and Golgi inheritance.

At the apex of complexity, we envision the GA of mammalian cells to be assembled in three stages, in which the cytoskeleton participates in a variable extent: the first flattens cisternae (which is actin-dependent); the second maintains the discrete stack structure in the tight parallel arrangement of a variable number of cisternae (which at the moment seems to be independent of cytoskeleton proteins), and the third maintains stacks together to produce the classical single ribbon-like Golgi structure (which is fully dependent on MTs). Variations in the relative contribution of each of these three steps could generate the diversity of GA arrangements observed in different biological systems.

Acknowledgements. We thank Michel Bornens, Inés Antón and members of our respective labs for their comments, as well as Sabrina Rivero and Francisco Lázaro-Diéguez for help with figures. The work carried out in our laboratories has been supported by grants from Ministerio de Educación y Ciencia (G.E. and R.M.R.), Junta de Andalucía (R.M.R.), and Distinció Award from the Generalitat de Catalunya (G.E).

References

Adams AE, Pringle JR (1984) Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. J Cell Biol 98: 934–945

Ameen NA, Figueroa Y, Salas PJ (2001) Anomalous apical plasma membrane phenotype in CK8-deficient mice indicates a novel role for intermediate filaments in the polarization of simple epithelia. J Cell Sci 114: 563–575

Ang AL, Folsch H, Koivisto UM, Pypaert M, Mellman I (2003) The Rab8 GTPase selectively regulates AP-1B-dependent basolateral transport in polarized Madin-Darby canine kidney cells. J Cell Biol 163: 339–350

Arasaki K, Taniguchi M, Tani K, Tagaya M (2006) RINT-1 regulates the localization and entry of ZW10 to the syntaxin 18 complex. Mol Biol Cell 17: 2780–2788

Au JS, Puri C, Ihrke G, Kendrick-Jones J, Buss F (2007) Myosin VI is required for sorting of AP-1B-dependent cargo to the basolateral domain in polarized MDCK cells. J Cell Biol 177: 103–114

Axelsson MA, Karlsson NG, Steel DM, Ouwendijk J, Nilsson T, Hansson GC (2001) Neutralization of pH in the Golgi apparatus causes redistribution of glycosyltransferases and changes in the O-glycosylation of mucins. Glycobiology 11: 633–644

Bard F, Casano L, Mallabiabarrena A, Wallace E, Saito K, Kitayama H, Guizzunti G, Hu Y, Wendler F, Dasgupta R, Perrimon N, Malhotra V (2006) Functional genomics reveals genes involved in protein secretion and Golgi organization. Nature 439: 604–607

Beck KA, Buchanan JA, Nelson WJ (1997) Golgi membrane skeleton: identification, localization and oligomerization of a 195 kDa ankyrin isoform associated with the Golgi complex. J Cell Sci 110 (Pt 10), 1239–1249

Becker B, Melkonian M (1996) The secretory pathway of protists: spatial and functional organization and evolution. Microbiol Rev 60: 697–721

Bennett V, Baines AJ (2001) Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. Physiol Rev 81: 1353–1392

Berezuk MA, Schroer TA (2007) Dynactin enhances the processivity of kinesin-2. Traffic 8: 124–129

Blancaflor EB (2002) The cytoskeleton and gravitropism in higher plants. J Plant Growth Regul 21: 120–136

Boevink P, Oparka K, Santa Cruz S, Martin B, Betteridge A, Hawes C (1998) Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. Plant J 15: 441–447

Bornens M (2002) Centrosome composition and microtubule anchoring mechanisms. Curr Opin Cell Biol 14: 25–34

Boutte Y, Vernhettes S, Satiat-Jeunemaitre B (2007) Involvement of the cytoskeleton in the secretory pathway and plasma membrane organisation of higher plant cells. Cell Biol Int 31: 649–654

Brandizzi F, Saint-Jore C, Moore I, Hawes C (2003) The relationship between endomembranes and the plant cytoskeleton. Cell Biol Int 27: 177–179

Brazer SC, Williams HP, Chappell TG, Cande WZ (2000) A fission yeast kinesin affects Golgi membrane recycling. Yeast 16: 149–166

Burgess TL, Skoufias DA, Wilson L (1991) Disruption of the Golgi apparatus with brefeldin A does not destabilize the associated detyrosinated microtubule network. Cell Motil Cytoskeleton 20: 289–300

Burkhardt JK, Echeverri CJ, Nilsson T, Vallee RB (1997) Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. J Cell Biol 139: 469–484

Buss F, Spudich G, Kendrick-Jones J (2004) Myosin VI: cellular functions and motor properties. Annu Rev Cell Dev Biol 20: 649–676

Camera P, da Silva JS, Griffiths G, Giuffrida MG, Ferrara L, Schubert V, Imarisio S, Silengo L, Dotti CG, Di Cunto F (2003) Citron-N is a neuronal Rho-associated protein involved in Golgi organization through actin cytoskeleton regulation. Nat Cell Biol 5: 1071–1078

Cao H, Weller S, Orth JD, Chen J, Huang B, Chen JL, Stamnes M, McNiven MA (2005) Actin and Arf1-dependent recruitment of a cortactin–dynamin complex to the Golgi regulates post-Golgi transport. Nat Cell Biol 7: 483–492

Carreno S, Engqvist-Goldstein AE, Zhang CX, McDonald KL, Drubin DG (2004) Actin dynamics coupled to clathrin-coated vesicle formation at the *trans*-Golgi network. J Cell Biol 165: 781–788

Caviston JP, Holzbaur EL (2006) Microtubule motors at the intersection of trafficking and transport. Trends Cell Biol 16: 530–537

Caviston JP, Ross JL, Antony SM, Tokito M, Holzbaur EL (2007) Huntingtin facilitates dynein/dynactin-mediated vesicle transport. Proc Natl Acad Sci USA 104: 10045–10050

Cohen D, Musch A, Rodriguez-Boulan E (2001) Selective control of basolateral membrane protein polarity by cdc42. Traffic 2: 556–564
Cole NB, Sciaky N, Marotta A, Song J, 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
Corthesy-Theulaz I, Pauloin A, Pfeffer SR (1992) Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. J Cell Biol 118: 1333–1345
Coulombe PA, Wong P (2004) Cytoplasmic intermediate filaments revealed as dynamic and multipurpose scaffolds. Nat Cell Biol 6: 699–706
Chabin-Brion K, Marceiller J, Perez F, Settegrana C, Drechou A, Durand G, Pous C (2001) The Golgi complex is a microtubule-organizing organelle. Mol Biol Cell 12: 2047–2060
Chang L, Goldman RD (2004) Intermediate filaments mediate cytoskeletal crosstalk. Nat Rev Mol Cell Biol 5: 601–613
Chen JL, Fucini RV, Lacomis L, Erdjument-Bromage H, Tempst P, Stamnes M (2005a) Coatomer-bound Cdc42 regulates dynein recruitment to COPI vesicles. J Cell Biol 169: 383–389
Chen JL, Lacomis L, Erdjument-Bromage H, Tempst P, Stamnes M (2004) Cytosol-derived proteins are sufficient for Arp2/3 recruitment and ARF/coatomer-dependent actin polymerization on Golgi membranes. FEBS Lett 566: 281–286
Chen JL, Xu W, Stamnes M (2005b) In vitro reconstitution of ARF-regulated cytoskeletal dynamics on Golgi membranes. Methods Enzymol 404: 345–358
Chen Y, Chen T, Shen S, Zheng M, Guo Y, Lin J, Baluska F, Samaj J (2006) Differential display proteomic analysis of Picea meyeri pollen germination and pollen-tube growth after inhibition of actin polymerization by latrunculin B. Plant J 47: 174–195
Dammermann A, Desai A, Oegema K (2003) The minus end in sight. Curr Biol 13: R614–R624
daSilva LL, Snapp EL, Denecke J, Lippincott-Schwartz J, Hawes C, Brandizzi F (2004) Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells. Plant Cell 16: 1753–1771
de Anda FC, Pollarolo G, Da Silva JS, Camoletto PG, Feiguin F, Dotti CG (2005) Centrosome localization determines neuronal polarity. Nature 436: 704–708
De Matteis MA, Di Campli A, Godi A (2005) The role of the phosphoinositides at the Golgi complex. Biochim Biophys Acta 1744: 396–405
De Matteis MA, Morrow JS (2000) Spectrin tethers and mesh in the biosynthetic pathway. J Cell Sci 113 (Pt 13): 2331–2343
Deacon SW, Serpinskaya AS, Vaughan PS, Lopez Fanarraga M, Vernos I, Vaughan KT, Gelfand VI (2003) Dynactin is required for bidirectional organelle transport. J Cell Biol 160: 297–301
Delacour D, Jacob R (2006) Apical protein transport. Cell Mol Life Sci 63: 2491–2505
DePina AS, Wollert T, Langford GM (2007) Membrane associated nonmuscle myosin II functions as a motor for actin-based vesicle transport in clam oocyte extracts. Cell Motil Cytoskeleton 64: 739–755
Devarajan P, Stabach PR, De Matteis MA, Morrow JS (1997) Na,K-ATPase transport from endoplasmic reticulum to Golgi requires the Golgi spectrin–ankyrin G119 skeleton in Madin Darby canine kidney cells. Proc Natl Acad Sci USA 94: 10711–10716
Devarajan P, Stabach PR, Mann AS, Ardito T, Kashgarian M, Morrow JS (1996) Identification of a small cytoplasmic ankyrin (AnkG119) in the kidney and muscle that binds beta I sigma spectrin and associates with the Golgi apparatus. J Cell Biol 133: 819–830
di Campli A, Valderrama F, Babia T, De Matteis MA, Luini A, Egea G (1999) Morphological changes in the Golgi complex correlate with actin cytoskeleton rearrangements. Cell Motil Cytoskeleton 43: 334–348

Dubois T, Paleotti O, Mironov AA, Fraisier V, Stradal TE, De Matteis MA, Franco M, Chavrier P (2005) Golgi-localized GAP for Cdc42 functions downstream of ARF1 to control Arp2/3 complex and F-actin dynamics. Nat Cell Biol 7: 353–364

Duran JM, Valderrama F, Castel S, Magdalena J, Tomas M, Hosoya H, Renau-Piqueras J, Malhotra V, Egea G (2003) Myosin motors and not actin comets are mediators of the actin-based Golgi-to-endoplasmic reticulum protein transport. Mol Biol Cell 14: 445–459

Efimov A, Kharitonov A, Efimova N, Loncarek J, Miller PM, Andreyeva N, Gleeson P, Galjart N, Maia AR, McLeod IX, Yates JR III, Maiato H, Khodjakov A, Akhmanova A, Kaverina I (2007) Asymmetric CLASP-dependent nucleation of noncentrosomal microtubules at the *trans*-Golgi network. Dev Cell 12: 917–930

Egea G, Lazaro-Dieguez F, Vilella M (2006) Actin dynamics at the Golgi complex in mammalian cells. Curr Opin Cell Biol 18: 168–178

Eng EW, Bettio A, Ibrahim J, Harrison RE (2007) MTOC reorientation occurs during FcgammaR-mediated phagocytosis in macrophages. Mol Biol Cell 18: 2389–2399

Estrada L, Caron E, Gorski JL (2001) Fgd1, the Cdc42 guanine nucleotide exchange factor responsible for faciogenital dysplasia, is localized to the subcortical actin cytoskeleton and Golgi membrane. Hum Mol Genet 10: 485–495

Fath KR (2005) Characterization of myosin-II binding to Golgi stacks in vitro. Cell Motil Cytoskeleton 60: 222–235

Fath KR, Burgess DR (1993) Golgi-derived vesicles from developing epithelial cells bind actin filaments and possess myosin-I as a cytoplasmically oriented peripheral membrane protein. J Cell Biol 120: 117–127

Finger FP, Novick P (2000) Synthetic interactions of the post-Golgi sec mutations of *Saccharomyces cerevisiae*. Genetics 156: 943–951

Fucini RV, Navarrete A, Vadakkan C, Lacomis L, Erdjument-Bromage H, Tempst P, Stamnes M (2000) Activated ADP-ribosylation factor assembles distinct pools of actin on golgi membranes. J Biol Chem 275: 18824–18829

Gao Y, Sztul E (2001) A novel interaction of the Golgi complex with the vimentin intermediate filament cytoskeleton. J Cell Biol 152: 877–894

Gao YS, Vrielink A, MacKenzie R, Sztul E (2002) A novel type of regulation of the vimentin intermediate filament cytoskeleton by a Golgi protein. Eur J Cell Biol 81: 391–401

Gillard BK, Clement R, Colucci-Guyon E, Babinet C, Schwarzmann G, Taki T, Kasama T, Marcus DM (1998) Decreased synthesis of glycosphingolipids in cells lacking vimentin intermediate filaments. Exp Cell Res 242: 561–572

Gillard BK, Thurmon LT, Harrell RG, Capetanaki Y, Saito M, Yu RK, Marcus DM (1994) Biosynthesis of glycosphingolipids is reduced in the absence of a vimentin intermediate filament network. J Cell Sci 107 (Pt 12): 3545–3555

Gloss A, Rivero F, Khaire N, Muller R, Loomis WF, Schleicher M, Noegel AA (2003) Villidin, a novel WD-repeat and villin-related protein from Dictyostelium, is associated with membranes and the cytoskeleton. Mol Biol Cell 14: 2716–2727

Godi A, Santone I, Pertile P, Devarajan P, Stabach PR, Morrow JS, Di Tullio G, Polishchuk R, Petrucci TC, Luini A, De Matteis MA (1998) ADP ribosylation factor regulates spectrin binding to the Golgi complex. Proc Natl Acad Sci USA 95: 8607–8612

Grigoriev I, Splinter D, Keijzer N, Wulf PS, Demmers J, Ohtsuka T, Modesti M, Maly IV, Grosveld F, Hoogenraad CC, Akhmanova A (2007) Rab6 regulates transport and targeting of exocytotic carriers. Dev Cell 13: 305–314

Haghnia M, Cavalli V, Shah SB, Schimmelpfeng K, Brusch R, Yang G, Herrera C, Pilling A, Goldstein LS (2007) Dynactin is required for coordinated bidirectional motility, but not for dynein membrane attachment. Mol Biol Cell 18: 2081–2089

Harada A, Takei Y, Kanai Y, Tanaka Y, Nonaka S, Hirokawa N (1998) Golgi vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein. J Cell Biol 141: 51–59

Harsay E, Schekman R (2007) Avl9p, a member of a novel protein superfamily, functions in the late secretory pathway. Mol Biol Cell 18: 1203–1219
Hawes C, Satiat-Jeunemaitre B (2005) The plant Golgi apparatus—going with the flow. Biochim Biophys Acta 1744: 93–107
Hayden SM, Wolenski JS, Mooseker MS (1990) Binding of brush border myosin I to phospholipid vesicles. J Cell Biol 111: 443–451
He CY (2007) Golgi biogenesis in simple eukaryotes. Cell Microbiol 9: 566–572
He CY, Ho HH, Malsam J, Chalouni C, West CM, Ullu E, Toomre D, Warren G (2004) Golgi duplication in *Trypanosoma brucei*. J Cell Biol 165: 313–321
Hehnly H, Stamnes M (2007) Regulating cytoskeleton-based vesicle motility. FEBS Lett 581: 2112–2118
Heimann K, Percival JM, Weinberger R, Gunning P, Stow JL (1999) Specific isoforms of actin-binding proteins on distinct populations of Golgi-derived vesicles. J Biol Chem 274: 10743–10750
Helfand BT, Chang L, Goldman RD (2003) The dynamic and motile properties of intermediate filaments. Annu Rev Cell Dev Biol 19: 445–467
Helfand BT, Mikami A, Vallee RB, Goldman RD (2002) A requirement for cytoplasmic dynein and dynactin in intermediate filament network assembly and organization. J Cell Biol 157: 795–806
Henderson GP, Gan L, Jensen GJ (2007) 3-D ultrastructure of *O. tauri*: electron cryotomography of an entire eukaryotic cell. PLoS ONE 2: e749
Heuvingh J, Franco M, Chavrier P, Sykes C (2007) ARF1-mediated actin polymerization produces movement of artificial vesicles. Proc Natl Acad Sci USA 104: 16928–16933
Hirose H, Arasaki K, Dohmae N, Takio K, Hatsuzawa K, Nagahama M, Tani K, Yamamoto A, Tohyama M, Tagaya M (2004) Implication of ZW10 in membrane trafficking between the endoplasmic reticulum and Golgi. EMBO J 23: 1267–1278
Holappa K, Munoz MT, Egea G, Kellokumpu S (2004) The AE2 anion exchanger is necessary for the structural integrity of the Golgi apparatus in mammalian cells. FEBS Lett 564: 97–103
Holappa K, Suokas M, Soininen P, Kellokumpu S (2001) Identification of the full-length AE2 (AE2a) isoform as the Golgi-associated anion exchanger in fibroblasts. J Histochem Cytochem 49: 259–269
Hoogenraad CC, Akhmanova A, Howell SA, Dortland BR, De Zeeuw CI, Willemsen R, Visser P, Grosveld F, Galjart N (2001) Mammalian Golgi-associated Bicaudal-D2 functions in the dynein–dynactin pathway by interacting with these complexes. EMBO J 20: 4041–4054
Hook P, Vallee RB (2006) The dynein family at a glance. J Cell Sci 119: 4369–4371
Hoppeler-Lebel A, Celati C, Bellett G, Mogensen MM, Klein-Hitpass L, Bornens M, Tassin AM (2007) Centrosomal CAP350 protein stabilises microtubules associated with the Golgi complex. J Cell Sci 120: 3299–3308
Hu Y, Zhong R, Morrison WH III, Ye ZH (2003) The *Arabidopsis* RHD3 gene is required for cell wall biosynthesis and actin organization. Planta 217: 912–921
Infante C, Ramos-Morales F, Fedriani C, Bornens M, Rios RM (1999) GMAP-210, A *cis*-Golgi network-associated protein, is a minus end microtubule-binding protein. J Cell Biol 145: 83–98
Jacob R, Heine M, Alfalah M, Naim HY (2003) Distinct cytoskeletal tracks direct individual vesicle populations to the apical membrane of epithelial cells. Curr Biol 13: 607–612
Jenna S, Caruso ME, Emadali A, Nguyen DT, Dominguez M, Li S, Roy R, Reboul J, Vidal M, Tzimas GN, Bosse R, Chevet E (2005) Regulation of membrane trafficking by a novel Cdc42-related protein in *Caenorhabditis elegans* epithelial cells. Mol Biol Cell 16: 1629–1639
Jordens I, Marsman M, Kuijl C, Neefjes J (2005) Rab proteins, connecting transport and vesicle fusion. Traffic 6: 1070–1077

Jung E, Fucini P, Stewart M, Noegel AA, Schleicher M (1996) Linking microfilaments to intracellular membranes: the actin-binding and vesicle-associated protein comitin exhibits a mannose-specific lectin activity. EMBO J 15: 1238–1246

Jurgens G (2005) Cytokinesis in higher plants. Annu Rev Plant Biol 56: 281–299

Kaksonen M, Toret CP, Drubin DG (2006) Harnessing actin dynamics for clathrin-mediated endocytosis. Nat Rev Mol Cell Biol 7: 404–414

Katsumoto T, Inoue M, Naguro T, Kurimura T (1991) Association of cytoskeletons with the Golgi apparatus: three-dimensional observation and computer-graphic reconstruction. J Electron Microsc (Tokyo) 40: 24–28

Kepes F, Rambourg A, Satiat-Jeunemaitre B (2005) Morphodynamics of the secretory pathway. Int Rev Cytol 242: 55–120

Kerkhoff E, Simpson JC, Leberfinger CB, Otto IM, Doerks T, Bork P, Rapp UR, Raabe T, Pepperkok R (2001) The Spir actin organizers are involved in vesicle transport processes. Curr Biol 11: 1963–1968

Keryer G, Di Fiore B, Celati C, Lechtreck KF, Mogensen M, Delouvee A, Lavia P, Bornens M, Tassin AM (2003a) Part of Ran is associated with AKAP450 at the centrosome: involvement in microtubule-organizing activity. Mol Biol Cell 14: 4260–4271

Keryer G, Witczak O, Delouvee A, Kemmner WA, Rouillard D, Tasken K, Bornens M (2003b) Dissociating the centrosomal matrix protein AKAP450 from centrioles impairs centriole duplication and cell cycle progression. Mol Biol Cell 14: 2436–2446

Kessels MM, Dong J, Leibig W, Westermann P, Qualmann B (2006) Complexes of syndapin II with dynamin II promote vesicle formation at the *trans*-Golgi network. J Cell Sci 119: 1504–1516

Kessels MM, Qualmann B (2004) The syndapin protein family: linking membrane trafficking with the cytoskeleton. J Cell Sci 117: 3077–3086

Kilmartin JV, Adams AE (1984) Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J Cell Biol 98: 922–933

Kim HS, Takahashi M, Matsuo K, Ono Y (2007) Recruitment of CG-NAP to the Golgi apparatus through interaction with dynein–dynactin complex. Genes Cells 12: 421–434

Kim S, Coulombe PA (2007) Intermediate filament scaffolds fulfill mechanical, organizational, and signaling functions in the cytoplasm. Genes Dev 21: 1581–1597

Kondylis V, Rabouille C (2003) A novel role for dp115 in the organization of tER sites in *Drosophila*. J Cell Biol 162: 185–198

Kondylis V, van Nispen tot Pannerden HE, Herpers B, Friggi-Grelin F, Rabouille C (2007) The golgi comprises a paired stack that is separated at G2 by modulation of the actin cytoskeleton through Abi and Scar/WAVE. Dev Cell 12: 901–915

Kostenko EV, Mahon GM, Cheng L, Whitehead IP (2005) The Sec14 homology domain regulates the cellular distribution and transforming activity of the Rho-specific guanine nucleotide exchange factor Dbs. J Biol Chem 280: 2807–2817

Kroschewski R, Hall A, Mellman I (1999) Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. Nat Cell Biol 1: 8–13

Kumemura H, Harada M, Omary MB, Sakisaka S, Suganuma T, Namba M, Sata M (2004) Aggregation and loss of cytokeratin filament networks inhibit Golgi organization in liver-derived epithelial cell lines. Cell Motil Cytoskeleton 57: 37–52

Kupfer A, Louvard D, Singer SJ (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

Ladinsky MS, Mastronarde DN, McIntosh JR, Howell KE, Staehelin LA (1999) Golgi structure in three dimensions: functional insights from the normal rat kidney cell. J Cell Biol 144: 1135–1149

Lanzetti L (2007) Actin in membrane trafficking. Curr Opin Cell Biol 19: 453–458

Larocca MC, Shanks RA, Tian L, Nelson DL, Stewart DM, Goldenring JR (2004) AKAP350 interaction with cdc42 interacting protein 4 at the Golgi apparatus. Mol Biol Cell 15: 2771–2781

Lazaro-Dieguez F, Colonna C, Cortegano M, Calvo M, Martinez SE, Egea G (2007) Variable actin dynamics requirement for the exit of different cargo from the *trans*-Golgi network. FEBS Lett 581: 3875–3881

Lazaro-Dieguez F, Jimenez N, Barth H, Koster AJ, Renau-Piqueras J, Llopis JL, Burger KN, Egea G (2006) Actin filaments are involved in the maintenance of Golgi cisternae morphology and intra-Golgi pH. Cell Motil Cytoskeleton 63: 778–791

Levine T, Rabouille C (2005) Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. Curr Opin Cell Biol 17: 362–368

Lu Z, Joseph D, Bugnard E, Zaal KJ, Ralston E (2001) Golgi complex reorganization during muscle differentiation: visualization in living cells and mechanism. Mol Biol Cell 12: 795–808

Luders J, Stearns T (2007) Microtubule-organizing centres: a re-evaluation. Nat Rev Mol Cell Biol 8: 161–167

Luna A, Matas OB, Martinez-Menarguez JA, Mato E, Duran JM, Ballesta J, Way M, Egea G (2002) Regulation of protein transport from the Golgi complex to the endoplasmic reticulum by CDC42 and N-WASP. Mol Biol Cell 13: 866–879

Malikov V, Cytrynbaum EN, Kashina A, Mogilner A, Rodionov V (2005) Centering of a radial microtubule array by translocation along microtubules spontaneously nucleated in the cytoplasm. Nat Cell Biol 7: 1213–1218

Malikov V, Kashina A, Rodionov V (2004) Cytoplasmic dynein nucleates microtubules to organize them into radial arrays in vivo. Mol Biol Cell 15: 2742–2749

Mardones GA, Snyder CM, Howell KE (2006) *Cis*-Golgi matrix proteins move directly to endoplasmic reticulum exit sites by association with tubules. Mol Biol Cell 17: 525–538

Marsh BJ, Mastronarde DN, Buttle KF, Howell KE, McIntosh JR (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

Matanis T, Akhmanova A, Wulf P, Del Nery E, Weide T, Stepanova T, Galjart N, Grosveld F, Goud B, De Zeeuw CI, Barnekow A, Hoogenraad CC (2002) Bicaudal-D regulates COPI-independent Golgi–ER transport by recruiting the dynein–dynactin motor complex. Nat Cell Biol 4: 986–992

Matas OB, Fritz S, Luna A, Egea G (2005) Membrane trafficking at the ER/Golgi interface: functional implications of RhoA and Rac1. Eur J Cell Biol 84: 699–707

Matas OB, Martinez-Menarguez JA, Egea G (2004) Association of Cdc42/N-WASP/Arp2/3 signaling pathway with Golgi membranes. Traffic 5: 838–846

Merrifield CJ (2004) Seeing is believing: imaging actin dynamics at single sites of endocytosis. Trends Cell Biol 14: 352–358

Merrifield CJ, Perrais D, Zenisek D (2005) Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. Cell 121: 593–606

Miki H, Okada Y, Hirokawa N (2005) Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol 15: 467–476

Miller DD, Scordilis SP, Hepler PK (1995) Identification and localization of three classes of myosins in pollen tubes of *Lilium longiflorum* and *Nicotiana alata*. J Cell Sci 108 (Pt 7): 2549–2563

Mizuno M, Singer SJ (1994) A possible role for stable microtubules in intracellular transport from the endoplasmic reticulum to the Golgi apparatus. J Cell Sci 107 (Pt 5): 1321–1331

Mogelsvang S, Gomez-Ospina N, Soderholm J, Glick BS, Staehelin LA (2003) Tomographic evidence for continuous turnover of Golgi cisternae in *Pichia pastoris*. Mol Biol Cell 14: 2277–2291

Mogensen MM, Malik A, Piel M, Bouckson-Castaing V, Bornens M (2000) Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. J Cell Sci 113 (Pt 17): 3013–3023

Mollenhauer HH, Morre DJ (1976) Cytochalasin B, but not colchicine, inhibits migration of secretory vesicles in root tips of maize. Protoplasma 87: 39–48
Montes de Oca G, Lezama RA, Mondragon R, Castillo AM, Meza I (1997) Myosin I interactions with actin filaments and *trans*-Golgi-derived vesicles in MDCK cell monolayers. Arch Med Res 28: 321–328
Moriyama Y, Nelson N (1989) H+-translocating ATPase in Golgi apparatus. Characterization as vacuolar H+-ATPase and its subunit structures. J Biol Chem 264: 18445–18450
Moseley JB, Goode BL (2006) The yeast actin cytoskeleton: from cellular function to biochemical mechanism. Microbiol Mol Biol Rev 70: 605–645
Mulholland J, Wesp A, Riezman H, Botstein D (1997) Yeast actin cytoskeleton mutants accumulate a new class of Golgi-derived secretary vesicle. Mol Biol Cell 8: 1481–1499
Musch A (2004) Microtubule organization and function in epithelial cells. Traffic 5: 1–9
Musch A, Cohen D, Kreitzer G, Rodriguez-Boulan E (2001) cdc42 regulates the exit of apical and basolateral proteins from the *trans*-Golgi network. EMBO J 20: 2171–2179
Musch A, Cohen D, Rodriguez-Boulan E (1997) Myosin II is involved in the production of constitutive transport vesicles from the TGN. J Cell Biol 138: 291–306
Nakamura N, Tanaka S, Teko Y, Mitsui K, Kanazawa H (2005) Four Na+/H+ exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. J Biol Chem 280: 1561–1572
Nebenfuhr A, Gallagher LA, Dunahay TG, Frohlick JA, Mazurkiewicz AM, Meehl JB, Staehelin LA (1999) Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. Plant Physiol 121: 1127–1142
Novick P, Botstein D (1985) Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell 40: 405–416
Omary MB, Coulombe PA, McLean WH (2004) Intermediate filament proteins and their associated diseases. N Engl J Med 351: 2087–2100
Oriolo AS, Wald FA, Ramsauer VP, Salas PJ (2007) Intermediate filaments: a role in epithelial polarity. Exp Cell Res 313: 2255–2264
Palokangas H, Ying M, Vaananen K, Saraste J (1998) Retrograde transport from the pre-Golgi intermediate compartment and the Golgi complex is affected by the vacuolar H+-ATPase inhibitor bafilomycin A1. Mol Biol Cell 9: 3561–3578
Pelletier L, Stern CA, Pypaert M, Sheff D, Ngo HM, Roper N, He CY, Hu K, Toomre D, Coppens I, Roos DS, Joiner KA, Warren G (2002) Golgi biogenesis in *Toxoplasma gondii*. Nature 418: 548–552
Percival JM, Froehner SC (2007) Golgi complex organization in skeletal muscle: a role for Golgi-mediated glycosylation in muscular dystrophies? Traffic 8: 184–194
Percival JM, Hughes JA, Brown DL, Schevzov G, Heimann K, Vrhovski B, Bryce N, Stow JL, Gunning PW (2004) Targeting of a tropomyosin isoform to short microfilaments associated with the Golgi complex. Mol Biol Cell 15: 268–280
Perrais D, Merrifield CJ (2005) Dynamics of endocytic vesicle creation. Dev Cell 9: 581–592
Picton JM, Steer MW (1981) Determination of secretory vesicle production rates by dictyosomes in pollen tubes of Tradescantia using cytochalasin D. J Cell Sci 49: 261–272
Praefcke GJ, McMahon HT (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol 5: 133–147
Prahlad V, Yoon M, Moir RD, Vale RD, Goldman RD (1998) Rapid movements of vimentin on microtubule tracks: kinesin-dependent assembly of intermediate filament networks. J Cell Biol 143: 159–170
Presley JF, Cole NB, Schroer TA, Hirschberg K, Zaal KJ, Lippincott-Schwartz J (1997) ER-to-Golgi transport visualized in living cells. Nature 389: 81–85
Preuss D, Mulholland J, Franzusoff A, Segev N, Botstein D (1992) Characterization of the *Saccharomyces* Golgi complex through the cell cycle by immunoelectron microscopy. Mol Biol Cell 3: 789–803

Prigozhina NL, Waterman-Storer CM (2004) Protein kinase D-mediated anterograde membrane trafficking is required for fibroblast motility. Curr Biol 14: 88–98

Pruyne D, Legesse-Miller A, Gao L, Dong Y, Bretscher A (2004) Mechanisms of polarized growth and organelle segregation in yeast. Annu Rev Cell Dev Biol 20: 559–591

Quintyne NJ, Gill SR, Eckley DM, Crego CL, Compton DA, Schroer TA (1999) Dynactin is required for microtubule anchoring at centrosomes. J Cell Biol 147: 321–334

Ralston E (1993) Changes in architecture of the Golgi complex and other subcellular organelles during myogenesis. J Cell Biol 120: 399–409

Ralston E, Lu Z, Ploug T (1999) The organization of the Golgi complex and microtubules in skeletal muscle is fiber type-dependent. J Neurosci 19: 10694–10705

Ralston E, Ploug T, Kalhovde J, Lomo T (2001) Golgi complex, endoplasmic reticulum exit sites, and microtubules in skeletal muscle fibers are organized by patterned activity. J Neurosci 21: 875–883

Rambourg A, Clermont Y (1986) Tridimensional structure of the Golgi apparatus in type A ganglion cells of the rat. Am J Anat 176: 393–409

Rambourg A, Jackson CL, Clermont Y (2001) Three dimensional configuration of the secretory pathway and segregation of secretion granules in the yeast *Saccharomyces cerevisiae*. J Cell Sci 114: 2231–2239

Rao MV, Engle LJ, Mohan PS, Yuan A, Qiu D, Cataldo A, Hassinger L, Jacobsen S, Lee VM, Andreadis A, Julien JP, Bridgman PC, Nixon RA (2002) Myosin Va binding to neurofilaments is essential for correct myosin Va distribution and transport and neurofilament density. J Cell Biol 159: 279–290

Rappleye CA, Paredez AR, Smith CW, McDonald KL, Aroian RV (1999) The coronin-like protein POD-1 is required for anterior–posterior axis formation and cellular architecture in the nematode *Caenorhabditis elegans*. Genes Dev 13: 2838–2851

Rehberg M, Kleylein-Sohn J, Faix J, Ho TH, Schulz I, Graf R (2005) Dictyostelium LIS1 is a centrosomal protein required for microtubule/cell cortex interactions, nucleus/centrosome linkage, and actin dynamics. Mol Biol Cell 16: 2759–2771

Ridgway ND, Dawson PA, Ho YK, Brown MS, Goldstein JL (1992) Translocation of oxysterol binding protein to Golgi apparatus triggered by ligand binding. J Cell Biol 116: 307–319

Ridley AJ (2006) Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. Trends Cell Biol 16: 522–529

Rios RM, Bornens M (2003) The Golgi apparatus at the cell centre. Curr Opin Cell Biol 15: 60–66

Rios RM, Sanchis A, Tassin AM, Fedriani C, Bornens M (2004) GMAP-210 recruits gamma-tubulin complexes to *cis*-Golgi membranes and is required for Golgi ribbon formation. Cell 118: 323–335

Robbins E, Gonatas NK (1964) Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell. J Histochem Cytochem 12: 704–711

Rodriguez ML, Brignoni M, Salas PJ (1994) A specifically apical sub-membrane intermediate filament cytoskeleton in non-brush-border epithelial cells. J Cell Sci 107 (Pt 11): 3145–3151

Rodriguez-Boulan E, Kreitzer G, Musch A (2005) Organization of vesicular trafficking in epithelia. Nat Rev Mol Cell Biol 6: 233–247

Rogalski AA, Bergmann JE, Singer SJ (1984) Effect of microtubule assembly status on the intracellular processing and surface expression of an integral protein of the plasma membrane. J Cell Biol 99: 1101–1109

Rohatgi R, Ho HY, Kirschner MW (2000) Mechanism of N-WASP activation by CDC42 and phosphatidylinositol 4,5-bisphosphate. J Cell Biol 150: 1299–1310

Ross JL, Wallace K, Shuman H, Goldman YE, Holzbaur EL (2006) Processive bidirectional motion of dynein–dynactin complexes in vitro. Nat Cell Biol 8: 562–570

Rossanese OW, Reinke CA, Bevis BJ, Hammond AT, Sears IB, O'Connor J, Glick BS (2001) A role for actin, Cdc1p, and Myo2p in the inheritance of late Golgi elements in *Saccharomyces cerevisiae*. J Cell Biol 153: 47–62

Rossanese OW, Soderholm J, Bevis BJ, Sears IB, O'Connor J, Williamson EK, Glick BS (1999) Golgi structure correlates with transitional endoplasmic reticulum organization in *Pichia pastoris* and *Saccharomyces cerevisiae*. J Cell Biol 145: 69–81

Rosso S, Bollati F, Bisbal M, Peretti D, Sumi T, Nakamura T, Quiroga S, Ferreira A, Caceres A (2004) LIMK1 regulates Golgi dynamics, traffic of Golgi-derived vesicles, and process extension in primary cultured neurons. Mol Biol Cell 15: 3433–3449

Roux A, Cappello G, Cartaud J, Prost J, Goud B, Bassereau P (2002) A minimal system allowing tubulation with molecular motors pulling on giant liposomes. Proc Natl Acad Sci USA 99: 5394–5399

Rozelle AL, Machesky LM, Yamamoto M, Driessens MH, Insall RH, Roth MG, Luby-Phelps K, Marriott G, Hall A, Yin HL (2000) Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP-Arp2/3. Curr Biol 10: 311–320

Rybakin V, Clemen CS (2005) Coronin proteins as multifunctional regulators of the cytoskeleton and membrane trafficking. Bioessays 27: 625–632

Rybakin V, Stumpf M, Schulze A, Majoul IV, Noegel AA, Hasse A (2004) Coronin 7, the mammalian POD-1 homologue, localizes to the Golgi apparatus. FEBS Lett 573: 161–167

Sahlender DA, Roberts RC, Arden SD, Spudich G, Taylor MJ, Luzio JP, Kendrick-Jones J, Buss F (2005) Optineurin links myosin VI to the Golgi complex and is involved in Golgi organization and exocytosis. J Cell Biol 169: 285–295

Saint-Jore CM, Evins J, Batoko H, Brandizzi F, Moore I, Hawes C (2002) Redistribution of membrane proteins between the Golgi apparatus and endoplasmic reticulum in plants is reversible and not dependent on cytoskeletal networks. Plant J 29: 661–678

Salas PJ, Rodriguez ML, Viciana AL, Vega-Salas DE, Hauri HP (1997) The apical submembrane cytoskeleton participates in the organization of the apical pole in epithelial cells. J Cell Biol 137: 359–375

Samaj J, Muller J, Beck M, Bohm N, Menzel D (2006) Vesicular trafficking, cytoskeleton and signalling in root hairs and pollen tubes. Trends Plant Sci 11: 594–600

Sandoval IV, Bonifacino JS, Klausner RD, Henkart M, Wehland J (1984) Role of microtubules in the organization and localization of the Golgi apparatus. J Cell Biol 99: 113s–118s

Saraste J, Goud B (2007) Functional symmetry of endomembranes. Mol Biol Cell 18: 1430–1436

Satiat-Jeunemaitre B, Cole L, Bourett T, Howard R, Hawes C (1996) Brefeldin A effects in plant and fungal cells: something new about vesicle trafficking? J Microsc 181: 162–177

Scales SJ, Pepperkok R, Kreis TE (1997) Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. Cell 90: 1137–1148

Sciaky N, Presley J, Smith C, Zaal KJ, Cole N, Moreira JE, Terasaki M, Siggia E, Lippincott-Schwartz J (1997) Golgi tubule traffic and the effects of brefeldin A visualized in living cells. J Cell Biol 139: 1137–1155

Schafer DA, Weed SA, Binns D, Karginov AV, Parsons JT, Cooper JA (2002) Dynamin2 and cortactin regulate actin assembly and filament organization. Curr Biol 12: 1852–1857

Schroer TA (2004) Dynactin. Annu Rev Cell Dev Biol 20: 759–779

Schulze E, Asai DJ, Bulinski JC, Kirschner M (1987) Posttranslational modification and microtubule stability. J Cell Biol 105: 2167–2177

Segui-Simarro JM, Austin JR II, White EA, Staehelin LA (2004) Electron tomographic analysis of somatic cell plate formation in meristematic cells of *Arabidopsis* preserved by high-pressure freezing. Plant Cell 16: 836–856

Shimmen T, Yokota E (2004) Cytoplasmic streaming in plants. Curr Opin Cell Biol 16: 68–72

Skoufias DA, Burgess TL, Wilson L (1990) Spatial and temporal colocalization of the Golgi apparatus and microtubules rich in detyrosinated tubulin. J Cell Biol 111: 1929–1937

Smythe E, Ayscough KR (2006) Actin regulation in endocytosis. J Cell Sci 119: 4589–4598

Soldati T, Schliwa M (2006) Powering membrane traffic in endocytosis and recycling. Nat Rev Mol Cell Biol 7: 897–908

Spelbrink RG, Nothwehr SF (1999) The yeast GRD20 gene is required for protein sorting in the *trans*-Golgi network/endosomal system and for polarization of the actin cytoskeleton. Mol Biol Cell 10: 4263–4281

Stamnes M (2002) Regulating the actin cytoskeleton during vesicular transport. Curr Opin Cell Biol 14: 428–433

Stankewich MC, Tse WT, Peters LL, Ch'ng Y, John KM, Stabach PR, Devarajan P, Morrow JS, Lux SE (1998) A widely expressed betaIII spectrin associated with Golgi and cytoplasmic vesicles. Proc Natl Acad Sci USA 95: 14158–14163

Stauber T, Simpson JC, Pepperkok R, Vernos I (2006) A role for kinesin-2 in COPI-dependent recycling between the ER and the Golgi complex. Curr Biol 16: 2245–2251

Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM (2006) Centrosome polarization delivers secretory granules to the immunological synapse. Nature 443: 462–465

Stow JL, Fath KR, Burgess DR (1998) Budding roles for myosin II on the Golgi. Trends Cell Biol 8: 138–141

Styers ML, Kowalczyk AP, Faundez V (2005) Intermediate filaments and vesicular membrane traffic: the odd couple's first dance? Traffic 6: 359–365

Styers ML, Salazar G, Love R, Peden AA, Kowalczyk AP, Faundez V (2004) The endo-lysosomal sorting machinery interacts with the intermediate filament cytoskeleton. Mol Biol Cell 15: 5369–5382

Takahashi M, Shibata H, Shimakawa M, Miyamoto M, Mukai H, Ono Y (1999) Characterization of a novel giant scaffolding protein, CG-NAP, that anchors multiple signaling enzymes to centrosome and the golgi apparatus. J Biol Chem 274: 17267–17274

Takahashi M, Yamagiwa A, Nishimura T, Mukai H, Ono Y (2002) Centrosomal proteins CG-NAP and kendrin provide microtubule nucleation sites by anchoring gamma-tubulin ring complex. Mol Biol Cell 13: 3235–3245

Tassin AM, Maro B, Bornens M (1985a) Fate of microtubule-organizing centers during myogenesis in vitro. J Cell Biol 100: 35–46

Tassin AM, Paintrand M, Berger EG, Bornens M (1985b) The Golgi apparatus remains associated with microtubule organizing centers during myogenesis. J Cell Biol 101: 630–638

Thyberg J, Moskalewski S (1985) Microtubules and the organization of the Golgi complex. Exp Cell Res 159: 1–16

Thyberg J, Moskalewski S (1993) Relationship between the Golgi complex and microtubules enriched in detyrosinated or acetylated alpha-tubulin: studies on cells recovering from nocodazole and cells in the terminal phase of cytokinesis. Cell Tissue Res 273: 457–466

Thyberg J, Moskalewski S (1999) Role of microtubules in the organization of the Golgi complex. Exp Cell Res 246: 263–279

Toivola DM, Tao GZ, Habtezion A, Liao J, Omary MB (2005) Cellular integrity plus: organelle-related and protein-targeting functions of intermediate filaments. Trends Cell Biol 15: 608–617

Trucco A, Polishchuk RS, Martella O, Di Pentima A, Fusella A, Di Giandomenico D, San Pietro E, Beznoussenko GV, Polishchuk EV, Baldassarre M, Buccione R, Geerts WJ,

Koster AJ, Burger KN, Mironov AA, Luini A (2004) Secretory traffic triggers the formation of tubular continuities across Golgi sub-compartments. Nat Cell Biol 6: 1071–1081

Tyska MJ, Mackey AT, Huang JD, Copeland NG, Jenkins NA, Mooseker MS (2005) Myosin-1a is critical for normal brush border structure and composition. Mol Biol Cell 16: 2443–2457

Uemura T, Yoshimura SH, Takeyasu K, Sato MH (2002) Vacuolar membrane dynamics revealed by GFP-AtVam3 fusion protein. Genes Cells 7: 743–753

Uitto J, Richard G, McGrath JA (2007) Diseases of epidermal keratins and their linker proteins. Exp Cell Res 313: 1995–2009

Valderrama F, Babia T, Ayala I, Kok JW, Renau-Piqueras J, Egea G (1998) Actin microfilaments are essential for the cytological positioning and morphology of the Golgi complex. Eur J Cell Biol 76: 9–17

Valderrama F, Duran JM, Babia T, Barth H, Renau-Piqueras J, Egea G (2001) Actin microfilaments facilitate the retrograde transport from the Golgi complex to the endoplasmic reticulum in mammalian cells. Traffic 2: 717–726

Valderrama F, Luna A, Babia T, Martinez-Menarguez JA, Ballesta J, Barth H, Chaponnier C, Renau-Piqueras J, Egea G (2000) The golgi-associated COPI-coated buds and vesicles contain beta/gamma-actin. Proc Natl Acad Sci USA 97: 1560–1565

Vallee RB, Varma D, Dujardin DL (2006) ZW10 function in mitotic checkpoint control, dynein targeting and membrane trafficking: is dynein the unifying theme? Cell Cycle 5: 2447–2451

Varma D, Dujardin DL, Stehman SA, Vallee RB (2006) Role of the kinetochore/cell cycle checkpoint protein ZW10 in interphase cytoplasmic dynein function. J Cell Biol 172: 655–662

Vidali L, McKenna ST, Hepler PK (2001) Actin polymerization is essential for pollen tube growth. Mol Biol Cell 12: 2534–2545

Wang C, JeBailey L, Ridgway ND (2002) Oxysterol-binding-protein (OSBP)-related protein 4 binds 25-hydroxycholesterol and interacts with vimentin intermediate filaments. Biochem J 361: 461–472

Warner CL, Stewart A, Luzio JP, Steel KP, Libby RT, Kendrick-Jones J, Buss F (2003) Loss of myosin VI reduces secretion and the size of the Golgi in fibroblasts from Snell's waltzer mice. EMBO J 22: 569–579

Watson P, Forster R, Palmer KJ, Pepperkok R, Stephens DJ (2005) Coupling of ER exit to microtubules through direct interaction of COPII with dynactin. Nat Cell Biol 7: 48–55

Wehland J, Henkart M, Klausner R, Sandoval IV (1983) Role of microtubules in the distribution of the Golgi apparatus: effect of taxol and microinjected anti-alpha-tubulin antibodies. Proc Natl Acad Sci USA 80: 4286–4290

Weiner OH, Murphy J, Griffiths G, Schleicher M, Noegel AA (1993) The actin-binding protein comitin (p24) is a component of the Golgi apparatus. J Cell Biol 123: 23–34

Welch MD, Mullins RD (2002) Cellular control of actin nucleation. Annu Rev Cell Dev Biol 18: 247–288

Welte MA (2004) Bidirectional transport along microtubules. Curr Biol 14: R525–R537

Yan X, Habedanck R, Nigg EA (2006) A complex of two centrosomal proteins, CAP350 and FOP, cooperates with EB1 in microtubule anchoring. Mol Biol Cell 17: 634–644

Yang YD, Elamawi R, Bubeck J, Pepperkok R, Ritzenthaler C, Robinson DG (2005) Dynamics of COPII vesicles and the Golgi apparatus in cultured *Nicotiana tabacum* BY-2 cells provides evidence for transient association of Golgi stacks with endoplasmic reticulum exit sites. Plant Cell 17: 1513–1531

Young J, Stauber T, del Nery E, Vernos I, Pepperkok R, Nilsson T (2005) Regulation of microtubule-dependent recycling at the *trans*-Golgi network by Rab6A and Rab6A′. Mol Biol Cell 16: 162–177

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