

# Cytoskeleton and Golgi-apparatus interactions: a two-way road of function and structure

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**Abstract:** The Golgi apparatus is the result of a complex and dynamic interaction between a large variety of molecules that determine its architecture, protein and lipid transports, and those that integrate signals from outside and inside the cell. The cytoskeleton facilitates the functional integration of all these processes. Association and coordination between microtubules and actin filaments, as well as their respective binding and regulatory proteins, are clearly necessary for Golgi structure and function. Protein sorting, membrane fission and fusion, and the motion of Golgi-derived transport carriers are all affected by both cytoskeleton elements.

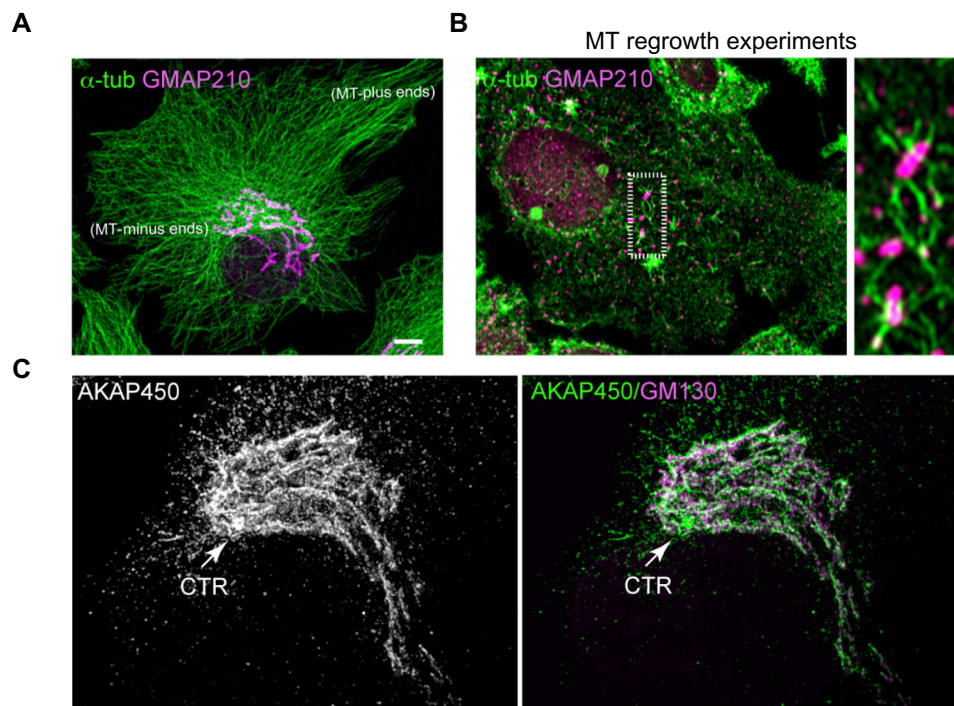
**Keywords:** cytoskeleton, Golgi apparatus, membrane trafficking, secretory pathway, actin, microtubules

## Introduction

The Golgi apparatus (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.<sup>1,2</sup> Unicellular green alga<sup>3</sup> and many protozoa like *Toxoplasma gondii*<sup>4</sup> and *Trypanosoma brucei*<sup>5,6</sup> contain a single pile of flattened cisternae aligned in parallel that is referred to as a dictyosome or Golgi stack.<sup>7</sup> In fungi,<sup>8,9</sup> plants,<sup>10,11</sup> or *Drosophila*,<sup>12</sup> many separate Golgi stacks are dispersed throughout the cytoplasm. In all these cases, each Golgi stack is associated with a single endoplasmic reticulum (ER) exit site, forming a secretory unit. In unpolarized nondividing mammalian cells, Golgi membranes are organized as a single-copy, ribbon-like shape and closely associated with the centrosome, which is usually located near the nucleus at the cell center.<sup>13,14</sup> These cells display a radial microtubule (MT) array, with plus-ends facing toward the cell cortex and minus-ends anchored at the centrosome (Figure 1A). Once positioned near the centrosome, Golgi membranes are linked laterally to form the ribbon-like membrane network, after which the Golgi ribbon may be directly anchored to the centrosome.<sup>15–17</sup> This organization is lost during cell division (mitosis), where a single Golgi ribbon first unlinks into individual stacks, which further undergo unstacking and vesiculation. These mitotic Golgi membranes are then partitioned between the two daughter cells, where they reassemble in a single Golgi ribbon after cytokinesis.<sup>18</sup> The remodeling of the MT cytoskeleton causes a large reorganization of the GA.<sup>19–22</sup> The cytoskeleton imposes the localization of the GA and largely participates in Golgi structure, cargo segregation at the *trans*-Golgi network (TGN), and subsequent production of transport

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**Figure 1** Microtubule–Golgi interaction.

**Notes:** (A) Immunofluorescence confocal image of RPE-1 cells double labeled for  $\gamma$ -tubulin (green) and the Golgi protein GMAP210 (magenta). Note the characteristic ribbon-like shape of the Golgi apparatus and its position close to the nucleus and minus-ends of microtubules (MTs). (B) MT-regrowth experiments after nocodazole treatment in RPE-1. Shortly after drug washout, MTs can be seen growing from dispersed Golgi elements.  $\alpha$ -Tubulin is in green and GMAP210 in magenta. Enlarged image of the outlined area is shown at right. (C) RPE-1 cells stained for AKAP450 (green) and GM130 (magenta) and visualized using an OMX superresolution microscope. Single labeling for AKAP450 is shown at left. White arrows indicate the centrosome (CTR). Bars, 10  $\mu$ m.

carriers (vesicles and/or tubules).<sup>17,23–26</sup> Which of these two cytoskeletal networks, MTs or actin filaments, is more critical for defining these properties depends on the cellular system.<sup>17,23–26</sup> In the following sections, we provide a general overview of the structural and functional consequences of the coupling between these two cytoskeletal elements and the GA. The interaction between the GA and intermediate filaments is poorly documented and not reviewed here, but has been in previous reports.<sup>24,27</sup>

## Microtubules in the structure and dynamics of the Golgi apparatus

### Structural relationship between the Golgi apparatus and microtubules

The essential role of the MT cytoskeleton in ensuring both the integrity of the GA as a single organelle and its pericentrosomal positioning has been acknowledged for a long time.<sup>28</sup> Both features, integrity and positioning, are lost when MT depolymerization is induced by such drugs as nocodazole (NZ). In the absence of MTs, the Golgi-ribbon fragments give rise to discrete Golgi elements, which appear dispersed throughout the cytoplasm.<sup>28,29</sup> The recent discovery

that MT nucleation occurs not only at the centrosome but also at the GA in many cell types has substantially changed the concept of the relationship between the GA and MTs. It is now widely accepted that centrosome-nucleated MTs are responsible for GA pericentrosomal positioning, whereas Golgi-derived MTs are required for ensuring the structural integrity of the Golgi ribbon.<sup>17,30</sup>

In the last decade, advances in microscope technology have boosted our knowledge of MT dynamics and their relationship with the GA. Three-dimensional electron microscopy analysis allowed the modeling of individual MTs and analysis of their relationship with cisternae in situ. Interestingly, MTs were found to run parallel to the first *cis*-cisterna of Golgi stacks over long distances. MTs also cross Golgi stacks at multiple points via noncompact regions and cisternal openings.<sup>31</sup> Time-lapse microscopy studies have revealed that in spite of GA's highly dynamic nature, global arrangement of the GA near the centrosome is relatively stable. Thin membrane tubules constantly form and detach from the lateral portions of almost stationary Golgi stacks.<sup>32–34</sup> After extending from the GA, some tubules break off and move along MTs toward the ER, where they collapse, thus delivering Golgi proteins to the ER.<sup>35</sup> Translocation of

transport intermediates from the ER to the GA also depends on MTs. In the absence of MTs, Golgi proteins recycle back to the ER. Then, they are exported into pre-Golgi intermediates that fail to move to the pericentrosomal region. Over long periods of time, these membrane elements organize themselves as stacks of cisternae and become fully competent for general secretion.<sup>29,36</sup> In a recent study, Tängemo et al employed laser nanosurgery to eliminate both Golgi complexes and centrosomes from living cells, and then they followed the de novo biogenesis of the GA.<sup>37</sup> By using time-lapse and correlative electron microscopy, they found that stacked and transport-competent Golgi stacks could be formed under these conditions. Overall, these studies support that the capacity to organize membranes as Golgi stacks is an intrinsic property of ER-derived membranes. This step does not require MTs. However, the fusion of stacks into a single organelle located at the cell center around the centrosome is an MT-dependent process.

## Microtubule motor proteins at the Golgi apparatus

A complete understanding of the mechanisms by which MTs influence the Golgi structure requires an understanding of how they contribute to membrane trafficking to and from the GA. Newly synthesized proteins and lipids at the ER are packed in transport carriers that move from peripherally located ER exit sites to the GA at the cell center. This anterograde transport occurs along MTs in a directional manner: transport carriers associate with MT plus-ends at the cell periphery and travel toward the minus-ends, where they fuse with the GA. This movement is powered by cytoplasmic dynein 1, which is the main minus-end-directed MT motor in eukaryotic cells.<sup>38–41</sup> The opposite movement, from the GA toward the ER, was reported to involve plus-end-directed molecular motors, such as kinesin 1 (KIF5B) and kinesin 2.<sup>42,43</sup> However, recent data point to a more complex scenario. By combining ribonucleic acid interference and automated image analysis followed by biochemical analysis, the role of different dynein subunits, and of kinesin 1 and 2 in membrane trafficking from the ER to the GA was examined.<sup>44,45</sup> They found that almost all known subunits of the cytoplasmic dynein 1 complex were required to maintain the Golgi structure. More specifically, a particular subset of dynein 1 containing light intermediate chain 1 appeared to be critical for ER-to-Golgi trafficking. Depletion of this subset did mimic a direct block of ER export. Intriguingly, knockdown of either kinesin 1 (KIF5B) or kinesin 2 caused an increase in the run length of membrane carriers in vivo,

suggesting that these motors can actually act as brakes on anterograde motility.<sup>45</sup> These data strongly support a direct role of kinesins in ER-to-Golgi transport, acting as negative regulators of this membrane pathway. This activity probably occurs through opposing the minus-end-directed force of dynein.<sup>45</sup>

Dynein and kinesins share structural similarities. They consist of two functional parts: a motor domain that moves along MTs by converting chemical energy (derived from adenosine triphosphate [ATP] hydrolysis) into directed motion, and a tail domain that mediates cargo interactions either directly or by specific accessory proteins.<sup>46</sup> How these motors are targeted to specific subcellular sites or how they bind their cargoes remains unclear. A candidate to mediate the interaction between dynein and membranes is dynactin, a multisubunit protein required for proper localization and activation of the molecular motor. Dynactin complex consists of eleven subunits,<sup>47,48</sup> among which the Arp1 filament p150<sup>Glued</sup> and p50/dynamitin are the best known. P150<sup>Glued</sup> is a coiled-coil dimeric protein that in addition to dynein binds both the Arp1 filament and MTs. On the other hand, p50/dynamitin plays an essential role in maintaining dynactin complex integrity.<sup>48</sup> Expression of either a dominant negative version of p150<sup>Glued</sup> or overexpression of p50/dynamitin leads to fragmentation and dispersion of the GA.<sup>49,50</sup> Under these conditions, trafficking of transport carriers between the ER and the GA is inhibited, as occurs in NZ-treated cells.<sup>32</sup> It has been suggested that binding of dynactin to transport carriers facilitates both proper formation of carriers and their motion to the GA.<sup>51</sup> This interaction is mediated by the p150<sup>Glued</sup> subunit of dynactin complex and Sec23p, a component of the COPII complex at the ER surface. Furthermore, the p150<sup>Glued</sup> subunit of dynactin also interacts with kinesin 2 through its nonmotor subunit KAP3.<sup>52</sup> Downregulation of KAP3 blocks the retrograde trafficking pathway from the GA to the ER and induces disorganization of Golgi membranes.<sup>42</sup> In summary, both kinesin 2 and dynein appear to bind the same dynactin subunit, suggesting a role for dynactin in regulating opposing motor activities and their processivity. Supporting this idea, both dynein and kinesin have been detected at the same membrane structures.<sup>52–56</sup>

The small guanosine triphosphatase (GTPase) Rab6 was also reported to play a major role in regulating membrane trafficking by recruiting MT motors to membranes.<sup>57–59</sup> It is known that members of the Rab6 family participate in some steps of MT-dependent vesicle transport from the TGN. When activated, Rab6 recruits the protein bicaudal D, which in turn recruits dynein–dynactin complexes to Rab6-positive

membranes, including Golgi elements.<sup>57–59</sup> It was suggested that these complexes might form part of a recycling pathway from the TGN back to the ER.<sup>60</sup> Other studies, however, pointed to the plasma membrane as the main target for the fusion of Rab6-containing vesicles. In this scenario, Rab6 would play a role in the constitutive secretory pathway by regulating both vesicle transport and targeting.<sup>61</sup> Supporting this hypothesis, bicaudal D1/2 was shown to interact with the plus-end-directed motor kinesin 1. Therefore, complex interactions involving dynein–dynactin, kinesin 1, Rab6, and bicaudal D1/2 appear to be required for Rab6-containing vesicles motion and targeting.<sup>59,61,62</sup> Altogether these data suggest that in order to ensure proper directionality of membrane elements, recruitment and coordination of MT motor activities should be finely regulated. Defining and integrating the specific contribution of each of these mechanisms to the overall regulation of Golgi dynamics deserve further investigation.<sup>47</sup>

## Golgins and microtubules at the Golgi apparatus

While it is clear that the minus-end-directed motor dynein is the main factor responsible for inward movement of Golgi membranes that results in its characteristic position near the centrosome, other nonmotor proteins appear to be important players in this process also. The best studied are golgin-160 and GMAP210.<sup>14,63,64</sup> Both of them are peripheral homodimeric coiled-coil proteins localized primarily at *cis*-Golgi cisternae. Depletion of each of them blocks Golgi positioning and yields dispersed in motile ministacks. Dispersed Golgi stacks in golgin-160- or GMAP210-depleted cells are competent for general protein transport to the cell surface, excluding an essential role for these proteins in membrane trafficking.<sup>63,64</sup>

Golgin-160 has been recently identified as the membrane receptor for dynein at the *cis*-Golgi.<sup>65</sup> It directly binds the dynein intermediate chain through a C-terminal coiled-coil segment in an Arf1-GTP-dependent manner. Golgin-160-dependent dynein recruitment was shown to be required for inward tracking movements of the Golgi membranes.<sup>65</sup> Once Golgi elements have reached the cell center, active anchoring or tethering to the centrosome might further maintain their pericentrosomal position. GMAP210 is a good candidate to carry out this activity, as when targeted to mitochondria, it induces their clustering around the centrosome.<sup>63,64</sup> GMAP210 has been shown to bind MT minus-ends and  $\gamma$ -tubulin.<sup>15</sup> GMAP210 has at least two membrane-targeting motifs located at the ends of the protein, both of which

are required to ensure its proper *cis*-Golgi localization and function.<sup>66,67</sup> The N-terminus of GMAP210 contains an amphipathic lipid-packing sensor motif, a sequence that has been reported to bind lipids and to act as a curvature sensor *in vitro*.<sup>66,68</sup> At the C-terminus, GMAP210 contains a GRIP-related ARF binding motif (it binds to membranes containing ARF1-GTP) that has been proposed to mediate ARF1-dependent recruitment to Golgi cisternae.<sup>69</sup> Interestingly, when ectopically expressed, the N-terminal binding motif is concentrated in the periphery of Golgi elements and between Golgi stacks, whereas the C-terminal domain appears uniformly distributed in the *cis*-cisternae of the GA. It has been proposed that by anchoring the surface of cisternae via its C-terminus and projecting its distal N-terminus to bind the rims or to stabilize tubular structures connecting neighboring *cis*-cisternae, GMAP210 would participate in homotypic fusion of *cis*-cisternae, thus contributing to ribbon formation around the centrosome.<sup>67</sup>

## Microtubule nucleation at the Golgi apparatus

The centrosome is the major site for MT nucleation and anchorage in proliferating and migrating cultured animal cells.<sup>70</sup> However, it has recently shown that the GA acts as an important secondary MT-organizing center (Figure 1B).<sup>17</sup> Indeed, it has been estimated that almost half of all cellular MTs originate from the GA in human RPE1 cells.<sup>71</sup> Chabin-Brion et al<sup>72</sup> showed for the first time that Golgi stacks were able to nucleate MTs in hepatic cells after NZ treatment. After drug washout, MTs apparently grew from scattered Golgi stacks. The authors further showed that purified Golgi membranes, which contained  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tubulin, supported MT nucleation *in vitro*. Additional evidence for a direct role for GA in MT nucleation was obtained from the analysis of centrosome-free cytoplasts. In these cytoplasts, radial MT arrays were able to form and become centralized in a dynein-dependent manner.<sup>73</sup> Strikingly, the GA appeared to be located at the center of centrosome-free cytoplasts, close to the MT aster.<sup>73</sup> The unequivocal demonstration of GA-associated MT nucleation came from experiments of laser ablation of the centrosome in primary cells.<sup>71</sup> By tracking polymerizing MTs *in vivo*, formation of MTs at Golgi stacks could be visualized either in normal or centrosome-lacking cells. MT nucleation at the GA was shown to require  $\gamma$ -tubulin complexes and the MT plus-end-binding CLASP proteins CLASP1 and CLASP2, which are recruited to the TGN through the interaction with the golgin GCC185. CLASPs are a family of MT-associated proteins that mainly bind

plus-ends of MTs, and in this way they contribute to their stabilization. It was then proposed that CLASPs at the TGN could stabilize preexisting MT seeds by coating them, thus preventing their depolymerization.

However, these studies did not identify the molecular machinery required for MT nucleation at the GA. Rivero et al<sup>74</sup> demonstrated that the centrosome GA-associated protein AKAP450 plays a key role in this process. AKAP450 is a giant coiled-coil protein of 450 kDa that is recruited to the *cis*-Golgi through its interaction with the golgin GM130 (Figure 1C). AKAP450 also interacts with  $\gamma$ -tubulin complexes,<sup>16,75</sup> and might in this way confer MT-nucleation capacity to the *cis*-Golgi compartment. Indeed, depletion of Golgi-associated AKAP450 fraction completely abolished MT nucleation at the GA. In addition, dissociation of AKAP450 from the GA by GM130 knockdown also dissociated MT-nucleation ability from the GA.<sup>74</sup> The GA-binding GM130-interacting domain was identified at the first N-terminal 300-aa coiled-coil stretch of the protein.<sup>76</sup> Expression of this domain dissociated AKAP450 from GA and abolished MT nucleation at the GA, confirming the involvement of AKAP450 in MT nucleation at the GA. Altogether, these data indicate that GM130-mediated recruitment of AKAP450 to the *cis*-Golgi compartment enable this organelle to generate its own MTs. Supporting this model, the *Drosophila* homolog of AKAP450 (CP309) has been recently reported to play a pivotal role in acentrosomal MT nucleation from Golgi outposts within terminal and primary branches of class IV dendritic arborization neurons.<sup>22</sup> MT nucleation from Golgi outposts in neurons required  $\gamma$ -tubulin in addition to AKAP450, and correlated with the extension and stability of terminal branches of dendrites.

Remarkably, two other centrosomal  $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC)-binding proteins – CDK5Rap2 and myomegalin – have recently been reported to bind AKAP450 and to localize at the *cis*-GA in an AKAP450-dependent manner.<sup>77–80</sup> CDK5RAP2 is a centrosome/GA protein that binds  $\gamma$ -tubulin complexes through the  $\gamma$ -TuRC-mediated nucleation activator motif. This motif directly binds to  $\gamma$ -TuRC and strongly stimulates its MT-nucleating activity.<sup>81</sup> CDK5Rap2 is required for  $\gamma$ -TuRC association with the centrosome,<sup>82</sup> suggesting that it could play a similar role at the GA. Myomegalin is a CDK5Rap2 paralog in vertebrates, highly expressed in muscle tissue, which was identified as a centrosome/Golgi protein associated with cyclic nucleotide phosphodiesterase.<sup>83</sup> Novel isoforms of myomegalin have recently been identified in nonmuscle cells. These myomegalin isoforms that localize predominantly to the *cis*-Golgi

interact with AKAP450,  $\gamma$ -tubulin complexes, and the MT plus-end-tracking protein EB1.<sup>78,80</sup> Interfering with the function of each of these proteins directly or indirectly perturbed MT nucleation at the GA, GA structural organization, and ER-to-GA transport.

Taken together, all these data support the idea that the *cis*-Golgi acquires the ability to nucleate MTs by acting as a preferential  $\gamma$ -TuRC-docking site, similarly to what it does at the centrosome. In addition, increasing evidence supports the idea that the MT-nucleating ability of the GA depends on multiprotein complexes similar to those operating at the pericentriolar material. In this scenario, AKAP450 appears as a key factor allowing the assembly of these multiprotein complexes at the surface of the *cis*-Golgi cisternae.<sup>17</sup> Since cytoplasmic pools of all these proteins exist, it is tempting to speculate that multiprotein complexes become competent for MT nucleation only after being assembled at the *cis*-Golgi membrane surface (or at the centrosome), in order to avoid random MT nucleation throughout the cytoplasm.

It should be taken into account that AKAP450 could also provide MT-formation/stabilization activities via additional mechanisms. For instance, AKAP450 also interacts with the dynactin subunit p150<sup>Glued</sup>.<sup>76,84</sup> The p150<sup>Glued</sup>-binding site is localized at the N-terminus of AKAP450 close to the GM130-interacting motif. When an AKAP450-truncated mutant containing both p150<sup>Glued</sup> and GM130-binding motifs was expressed, it targeted the GA and displaced endogenous protein from the Golgi. Interestingly, this truncated mutant also decorated cytoplasmic MTs, suggesting that AKAP450 could bind MTs through binding to p150<sup>Glued</sup>.<sup>76</sup> These results, together with the finding that blocking dynein/dynactin interferes with GA-based MT nucleation at the GA,<sup>74</sup> suggest that AKAP450 could participate in several ways in MT formation at the GA.

Newly nucleated MTs at the *cis*-GA need to be stabilized in order to prevent their disassembly. This would allow the formation of the dense meshwork of short MTs at the GA area that is found in the majority of cell types. As mentioned before, CLASP proteins seem to be essential in this process. Therefore, CLASPs and AKAP450-dependent multiprotein complexes should cooperate in the formation of this population of MTs.

How this cooperation occurs is far from clear. AKAP450 and CLASPs localize at opposite sides of Golgi stacks: AKAP450 localizes at the *cis*-Golgi, whereas CLASPs associate to the TGN.<sup>71,74</sup> It was first proposed that very short MT fragments generated at the *cis*-Golgi would be stabilized by CLASP proteins at the TGN, allowing them to serve as seeds

for polymerization. Alternatively, MT seeds could elongate from the *cis* face of Golgi stacks and subsequently be stabilized at their plus ends. This would generate a meshwork of short MTs within, and rather parallel to, the Golgi cisternae that may favor tangential linking and fusion of Golgi stacks into a Golgi ribbon. Additionally, CLASPs might stabilize MTs nucleated from the *cis*-Golgi that extends through the TGN toward the cell periphery, specially in migrating cells, in which Golgi-associated MT arrays are oriented toward the cell front.<sup>17,30</sup>

Other proteins can also cooperate in the stabilization of Golgi-based MTs. Among them, CAP350 is a centrosomal protein initially described as an MT-anchoring protein at the centrosome.<sup>85</sup> It has been reported that CAP350 contributes to the maintenance of Golgi-ribbon positioning and integrity by specifically stabilizing a subset of MTs associated with the GA.<sup>86</sup> Intriguingly, the suppressor tumor protein PTTG1/securin has been recently found to be associated with the GA in an MT-independent manner.<sup>87</sup> It seems to exist in a complex with AKAP450, GM130, and  $\gamma$ -tubulin. Depletion of PTTG1 resulted in a delay of both centrosomal and GA-based MT nucleation. Based on these results, it has been proposed that PTTG1/securin might have a role as a chaperone contributing to the formation and stability of MT-nucleating complexes.<sup>87</sup>

## Functions of Golgi-nucleated microtubules in cell dynamics

Several recent studies have investigated the functions of Golgi-derived MTs. They have revealed that Golgi-based MTs participate in a wide range of cellular processes, including maintenance of Golgi morphology and integrity, Golgi reassembly after mitosis, directional cell migration, polarized secretion and ciliogenesis.<sup>71,76,88–90</sup>

First insights on this issue came from live-cell imaging analysis of Golgi re-assembly during NZ recovery.<sup>90</sup> It was shown that this assembly takes place in two different phases: first Golgi ministacks fuse into larger elements at the cell periphery, and then these peripheral clusters are transported to the cell center, where they tangentially connect to form a single-membrane unit. Golgi-derived MTs were essential for the first phase, since abolition of Golgi-nucleation ability led to a selective defect of peripheral clustering and initial fusion of dispersed Golgi elements. Under these circumstances, a circular GA surrounding the centrosome formed, but it was highly fragmented, as revealed by FRAP (fluorescence recovery after photobleaching) experiments. These results indicated that Golgi elements can be translocated toward

the cell center in the absence of Golgi-nucleated MTs, but they are unable to fuse into a continuous Golgi ribbon.<sup>76,90</sup> In a more physiological context, such as cell division, Golgi-nucleated MTs were shown to be responsible for Golgi reassembly at the end of mitosis. In the presence of a dominant negative mutant of AKAP450, Golgi stacks accumulated at spindle poles during telophase, but they remained small and fragmented, indicating that Golgi-associated AKAP450-dependent MT nucleation is essential to bring together emerging Golgi stacks in the course of postmitotic assembly.<sup>88</sup> Fusion of Golgi stacks into a polarized Golgi ribbon is a complex process that implies both lateral linking and fusion of homotypic cisternae. Golgi-nucleated MTs that grow tangentially to stacks might participate in this process by allowing Golgi stacks to properly align, thus facilitating successive linking and fusion events.

One striking feature of Golgi-nucleated MTs is their asymmetry, which could be relevant for such cellular processes as directional secretion or cell migration. Indeed, polarized post-Golgi trafficking was impaired in conditions where Golgi MT-nucleation ability was abolished.<sup>76,90</sup> In the same way, cells lacking Golgi-derived MTs migrated slower in wound-healing assays in spite of proper coordinated reorientation of both the centrosome and the GA toward the leading edge.<sup>76,90</sup> Since in the absence of Golgi-nucleated MTs, Golgi stacks localized around the centrosome but remained unfused, one might conclude that Golgi-ribbon integrity is more important for these processes than its pericentrosomal position. The interference with AKAP450 function supported the view that the pericentrosomal position of the Golgi ribbon is a more determinant factor for cell polarity and directional migration than Golgi-ribbon integrity.<sup>76</sup> Indeed, disruption of Golgi-centrosome association induced by the expression of the N-terminus of AKAP450 had a stronger negative effect on cell polarity and migration than simply inhibiting MT nucleation. This is in agreement with previous evidence that motile cells require a polarized Golgi complex in proximity to the centrosome for proper directional post-Golgi trafficking and directional cell migration.<sup>91</sup>

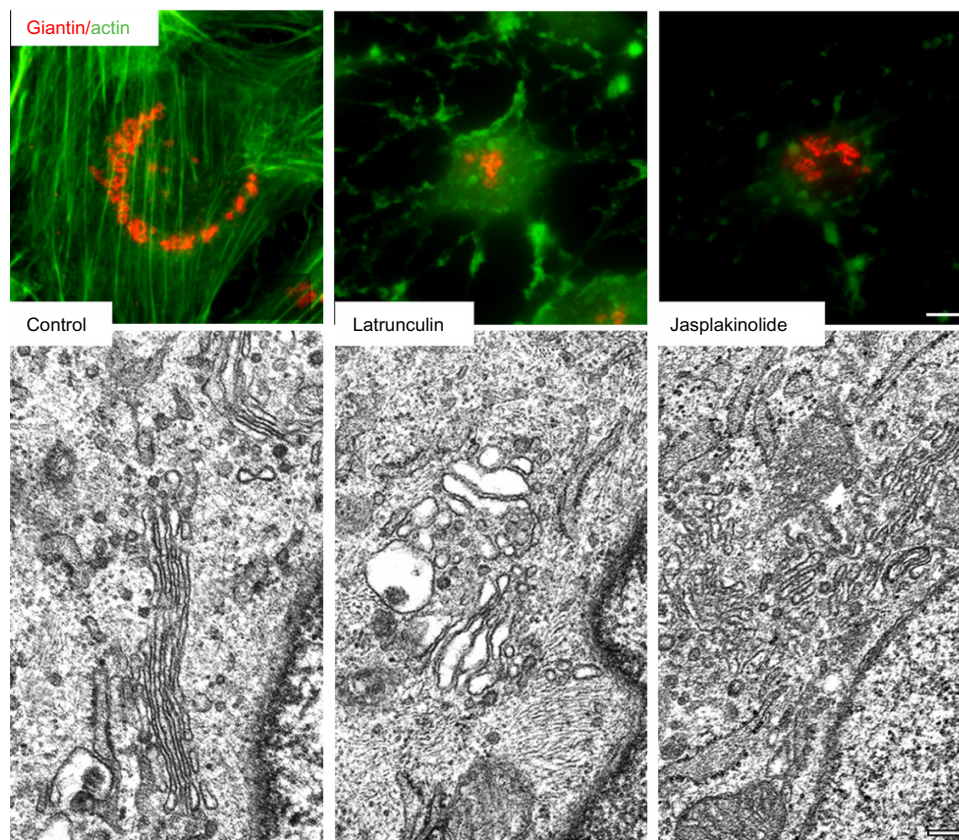
## The actin-based cytoskeleton and the Golgi apparatus

The first experimental evidence that actin and the Golgi may interact occurred when Golgi membranes and Golgi-derived vesicles contained actin and actin-binding proteins.<sup>92</sup> Furthermore, the Golgi invariably compacted when cells were treated with naturally occurring substances that perturbed the actin organization and its dynamics, which mainly include

cytochalasins, latrunculins, jasplakinolide, and botulinum toxins and are known generically as actin toxins or actin drugs<sup>93–97</sup> (Figure 2, top row). At an ultrastructural level, the compacted Golgi was seen to depend on whether actin drugs depolymerized or stabilized actin filaments, giving rise respectively to dilatation or fragmentation/perforation of cisternae (Figure 2, bottom row). Moreover, these ultrastructural impairments occurred in an MT-independent manner, which ruled out synergic cooperation between MTs and actin filaments controlling the shape and integrity of Golgi cisternae.<sup>94</sup> Golgi compactness is consistently seen when actin partners present at the Golgi complex are perturbed, such as after the depletion of the Arp2/3 activator WHAMM (WASp homolog associated with actin, Golgi membranes, and MTs),<sup>98</sup> cortactin,<sup>99</sup> or myosin 18A, an unconventional myosin that connects filamentous actin to the phosphatidylinositol 4-phosphate (PI4P)-binding protein GOLPH3.<sup>100,101</sup> However, in some cases, interference with the actin machinery produces fragmentation (and dispersion) of the Golgi, which occurs after the depletion or constitu-

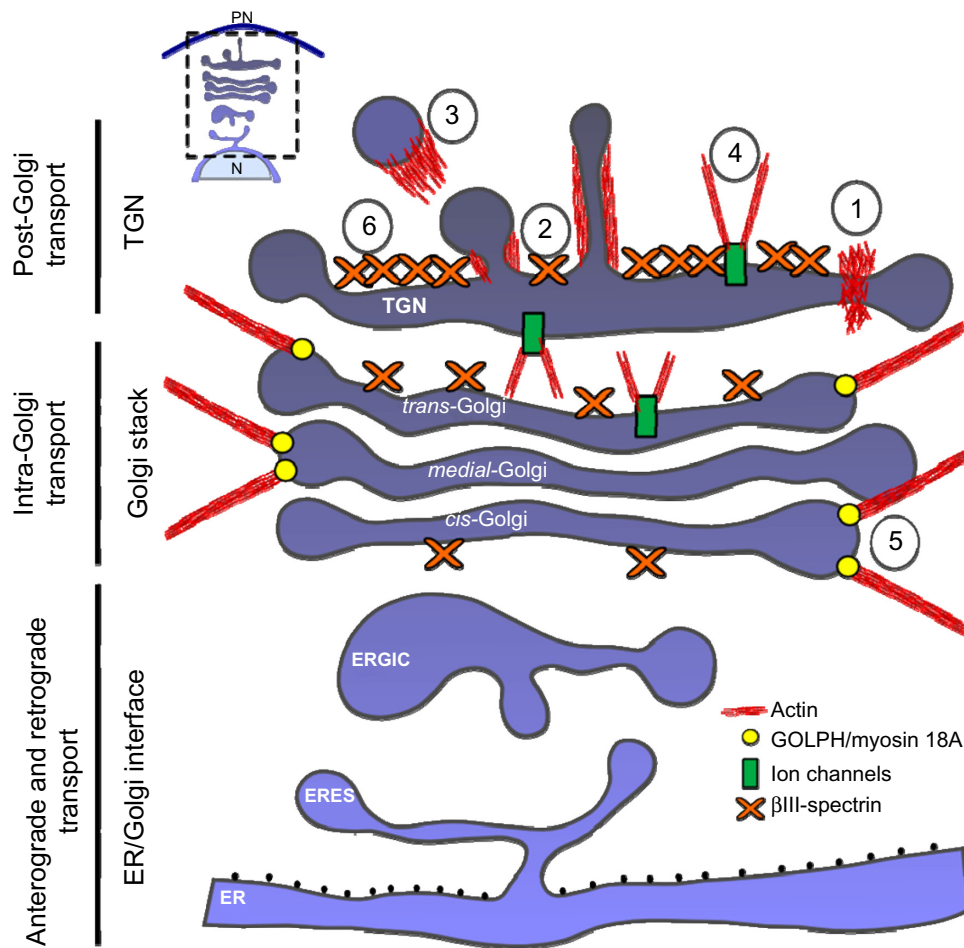
tive activation of actin nucleator formin family members mDia, the formin-like 1/FMNL1, and INF2.<sup>102–104</sup> Taken together, these findings reinforce the notion that the proper regulation of actin at the Golgi is necessary to maintain the structural integrity of the GA. In Figure 3 are summarized the different steps in which actin and actin-binding/regulatory proteins have been firmly established, and these are further developed here.

By analogy with erythrocytes, the Golgi-associated spectrin skeleton could act as an extended, two-dimensional interactive platform on the cytoplasmic surface of cisternae, regulating its shape and transport functions.<sup>105–109</sup> While mammalian red blood cells contain only one type of spectrin tetramer ( $\alpha\beta$ I subunits), nucleated cells contain numerous isoforms of both subunits, with  $\beta$ III spectrin being localized to the Golgi.<sup>110,111</sup> Other isoforms of the spectrin-based cytoskeleton components typically present in the plasma membrane of red blood cells have also been localized in the Golgi, such as  $\beta$ - and  $\gamma$ -actin,<sup>97</sup> ankyrins Ank<sub>G119</sub> and Ank<sub>195</sub>,<sup>112–114</sup> protein 4.1B,<sup>115</sup> anion exchanger AE2,<sup>116,117</sup> and



**Figure 2** Actin–Golgi interaction I.

**Notes:** Immunofluorescence microscopy image of NRK cells double-labeled for filamentous actin (with phalloidin–fluorescein isothiocyanate [green]) and for the Golgi-matrix protein giantin (with antibodies to giantin [red]). Untreated cells (control) and cells treated with antiactin agents, such as latrunculin (actin depolymerizer) or jasplakinolide (actin stabilizer), are shown. Notice the characteristic compaction of the Golgi caused by both toxins. However, under the electron microscope, their effects on the Golgi ultrastructure are very different: latrunculin leads to the swelling of cisternae, while jasplakinolide causes their fragmentation. Bars, 5 and 0.2  $\mu$ M, respectively.



**Figure 3** Actin–Golgi interaction II.

**Notes:** Diagram of the secretory membrane trafficking pathways and events in which actin is known to participate. Actin filaments, their polymerization, and dynamics could act as a force for the scission (1), pulling (2), and propelling (3) of the transport carrier generated in cisternae, and for maintaining the flattened shape of cisternae through the regulation of the activity on some ion pumps/channels (4) and/or being part of the spectrin-based cytoskeleton (6), and to keep the Golgi ribbon extended (5). Modified from Springer, *Histochemistry and Cell Biology*. Actin acting at the Golgi. 2013;140(3):347–360. Egea G, Serra-Peinado C, Salcedo-Sicilia L, Gutiérrez-Martínez E. Copyright © Springer-Verlag Berlin Heidelberg 2013, with kind permission of Springer Science+Business Media.<sup>24</sup>

**Abbreviations:** TGN, *trans*-Golgi network; ERGIC, endoplasmic reticulum–Golgi intermediate compartment; ERES, ER exiting sites.

tropomyosin.<sup>118</sup>  $\beta$ III spectrin maintains Golgi architecture, since its functional interference or knockdown causes fragmentation and dilation of Golgi membranes.<sup>110,119</sup> It is possible that the Golgi fragmentation is produced by the loss of the direct interaction of  $\beta$ III spectrin with the dynein–dynactin motor-complex subunit Arp1,<sup>120</sup> and distal cisternae swelling is caused by alterations in the activity of ionic channels, in the mechanical stability of cisternae, or both. Strikingly, actin toxins did not perturb the localization of  $\beta$ III spectrin at the Golgi, which indicates that actin dynamics do not participate in the association of  $\beta$ III spectrin with Golgi membranes, but PI4P was crucial in such interaction.<sup>110</sup> The ultrastructural alterations caused by actin drugs<sup>94</sup> and the depletion of  $\beta$ III spectrin<sup>110</sup> indicate that they provide mechanical stability to cisternae. Ion-regulatory molecules, such as vacuolar H<sup>+</sup>-ATPase<sup>121</sup> and cation exchangers (Na<sup>+</sup>/H<sup>+</sup> exchangers),<sup>122</sup>

either resident in the Golgi or in transit to the plasma membrane, could contribute to this cisternae mechanical stability, finely regulating intra-Golgi ion concentration and pH homeostasis. In this, sense actin filament-severing protein ADF/cofilin participates in cargo sorting at the TGN by activating the calcium ATPase 1 (SPCA1).<sup>123–125</sup> In addition, cisternae swelling induced by latrunculin is accompanied by a rise in the intra-Golgi pH.<sup>94</sup> Therefore, actin seems to regulate the activity of some ionic regulatory proteins present in Golgi membranes, similar to what occurs at the plasma membrane.<sup>126</sup>

### Actin in the sorting, biogenesis, and motion of transport carriers at the Golgi

A key aspect in the structure of polarized cells is the maintenance of polarized molecular organization. This is based on



highly specific sorting machinery at the exit of the TGN.<sup>127</sup> Cytoskeleton elements form part of this machinery, and the integrity of actin filaments is necessary for efficient delivery of some proteins destined for the apical or the basolateral plasma membrane domains in both polarized and unpolarized cells, but not for the transport of lipid raft-associated proteins.<sup>128</sup>

### Rho GTPases and actin-polymerization molecular machinery

The coupling between Golgi-associated actin polymerization and membrane-elongation and fission reactions must be subjected to control to prevent the structural and functional collapse of the Golgi. Rho GTPases and downstream effectors in Golgi membranes are essential to this aim. Rho GTPases cycle between active GTP-bound and inactive GDP-bound forms with the participation of guanine nucleotide-exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide-dissociation inhibitors. Cdc42, which was the first Rho GTPase to be localized in the Golgi,<sup>129–134</sup> affects the ER–Golgi interface and post-Golgi intracellular trafficking.<sup>135</sup> Constitutively active and inactive Cdc42 mutants block the ER-to-Golgi transport of anterograde cargo (VSV-G).<sup>134</sup> The overexpression and activation of Cdc42<sup>131</sup> or the knockdown of Cdc42 GAP ARHGAP21 (also known as ARHGAP10)<sup>136</sup> inhibits the Golgi-to-ER transport of retrograde cargo (Shiga toxin). Cdc42 directly binds to the coatamer or COPI system, particularly the  $\gamma$ COPI subunit, recruiting N-WASP and Arp2/3 to Golgi membranes. A receptor for cargo containing the dilysine motif in the COOH terminal, p23 competes with Cdc42 for binding to the  $\gamma$ COPI subunit. Cargo loading by p23 disrupts Cdc42– $\gamma$ COP interaction and recruits dynein to promote dynein-dependent ER-to-Golgi transport.<sup>137</sup> Therefore, Cdc42 coordinates actin- and MT-dependent motility of transport carriers at the ER–Golgi interface.<sup>138</sup> In post-Golgi trafficking, the expression of constitutively active or inactive Cdc42 mutants slows the exit of basolateral protein markers and accelerates the exit of apically destined ones.<sup>139–141</sup> ARHGAP21/10 and Cdc42 GEFs Fgd1 and Dbs are also present in Golgi membranes regulating post-Golgi vesicular transport.<sup>142–144</sup>

Besides Cdc42, recent evidence indicates that other Rho GTPases are working at the Golgi. Therefore, we have 1) RhoA with the actin nucleator mDia or mammalian diaphanous-related formin 1,<sup>104</sup> which accelerates actin nucleation and elongation by interacting with barbed ends of microfilaments, and the neuronal RhoA-associated protein citron-N,<sup>145</sup> which assembles together the Rho effector ROCK

II and the actin-binding, neuron-specific, protein profilin IIa. 2) RhoD with the WAS (Wiskott–Aldrich syndrome) protein homolog associated with actin, Golgi membranes, and MTs (WHAMM),<sup>146</sup> which plays a role in actin nucleation, Golgi-membrane association, and MT binding. WHAMM is a nucleation-promoting factor that regulates the actin-related protein 2/3 complex. The activated complex initiates growth of new actin filaments by binding to existing actin filaments. WHAMM also functions in regulation of transport from the ER to the Golgi complex and in maintenance of the Golgi complex near the centrosome. And finally we have 3) Rac1 and its exchange factor  $\beta$ -PIX with the clathrin heavy chain-binding protein CYFIP/Sra/PIR121.<sup>147</sup> RhoD-WHAMM and the ARF1-primed Rac1-CYFIP/Sra/PIR121 protein complexes stimulate the Arp2/3-induced actin polymerization at the Golgi and vesicle biogenesis.<sup>98,147</sup> The ROCK/LIM kinase (LIMK) signaling pathway and its substrate cofilin are necessary for apical cargoes.<sup>148,149</sup> RhoA GEF-H1 interacts with exocyst component Sec5, which in turn activates RhoA regulating post-Golgi trafficking and assembly of other exocyst components.<sup>150</sup>

The presence in Golgi membranes of molecular components that trigger actin polymerization with those that control vesicular budding and fission suggests intimate molecular coupling between them, which is similar to that observed during endocytosis.<sup>151</sup> Actin assembly provides the structural support and generates force for the formation of transport carriers in the lateral portions of Golgi membranes, which is facilitated by actin nucleators and accompanied by the mechanical activity of actin motors (myosins). In accordance with this idea, Arp2/3, mDia, formin-like 1/FMNL1 and INF2, and Spir1 are all present in the Golgi.<sup>132,152–154</sup> At the TGN, there is a functional coupling between dynamin-mediated membrane fission and Arp2/3-mediated actin-based mechanisms.<sup>152,154–158</sup> Interference with dynamin 2/cortactin or dynamin 2/syndapin 2/cortactin blocks post-Golgi protein transport.<sup>155,159</sup> As indicated earlier, in early Golgi compartments there is a functional connection between actin polymerization governed by Cdc42, coatamer (COPI)-mediated transport-carrier formation, and MT motor-mediated motion. WASH (WAS protein and SCAR homolog) is another Arp2/3 activator that regulates cation independent-mannose phosphate receptor (CI-MPR) trafficking from endosomes to the Golgi, forming an endosomal subdomain containing Arp2/3, F-actin, tubulin, and retromer components.<sup>160</sup> The local fine regulation of actin dynamics on the transport-carrier assembly represents an early step that precedes its scission taking place in the

lateral rims of cisternae for subsequent switching to MT tracks for motion.

On the other hand, actin nucleation/polymerization activity associated with Arp2/3 could give also 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.<sup>161</sup> After the overexpression of phosphatidylinositol 5-kinase, actin tails have been observed in raft-enriched TGN-derived vesicles.<sup>158</sup> An *in vitro* approach in liposomes showed actin polymerization occurring after the recruitment of the activated form of ARF1 around liposomes. This actin polymerization was dependent on Cdc42 and N-WASP, and resulted in the formation of actin comets, which pushed the ARF1-containing liposomes forward.<sup>162</sup> However, actin comet tails do not seem to be an efficient mechanism to provide precise directionality for transport carriers. Also, an actin comet tail-like mechanism could easily provide brief local force to facilitate the final separation of the transport carrier at the lateral rims of Golgi cisternae similarly to what happens during endocytosis,<sup>163,164</sup> and/or for its translocation to closely arranged MT tracks.<sup>165</sup>

### Myosin motors and actin itself as molecular and mechanical constraints

In addition to actin polymerization, myosins also generate a force that can selectively couple protein sorting and transport-carrier biogenesis and motility. Class I myosin is a monomeric, nonprocessive motor that binds to Golgi membranes and is present on apical Golgi-derived vesicles of polarized cells.<sup>166–170</sup> Myosin Ib together with actin polymerization have recently been shown to participate in membrane remodeling to form tubular transport carriers at the TGN directed to endosomes and the plasma membrane.<sup>166,171</sup> It has been hypothesized that myosin Ib spatially controls actin assembly at the TGN, interacting with F-actin via its motor domain and at the membrane via its PH domain. Such interaction generates a force concomitantly with the polymerization of actin, leading to membrane-curvature changes.<sup>171,172</sup> Coudrier and Almeida suggested coordination between myosin Ib and nonmuscle myosin II for the scission of tubular carriers at the TGN.<sup>171</sup> Myosin Ic controls the delivery of GPI-linked cargo proteins to the cell surface from the endosomal recycling compartment,<sup>173</sup> but this does not seem to be the case for myosin Ib, either at the TGN or in endosomes.<sup>166</sup>

Nonmuscle myosin II is another nonprocessive motor that interacts with Golgi membranes<sup>174</sup> and mediates Golgi-to-ER and post-Golgi protein transport.<sup>175–178</sup> It has been hypothesized that myosin II is tethered to the cisterna by its

tail and to actin filaments by its motor head. Its subsequent motion along actin filaments could provide the force needed to extend Golgi-derived membranes away from the cisterna, which could facilitate the functional coupling of membrane scission protein(s), leading to the release of the transport carrier. In this respect, myosin II forms a complex with Rab6, which drives its localization to Golgi membranes and controls the fission of anterograde and retrograde Rab6 transport carriers.<sup>179</sup> The Golgi-associated tropomyosin isoform<sup>118</sup> could stabilize short actin filaments formed locally in the cisternae lateral rims during vesicle biogenesis. These short actin filaments together with myosin II could equivalently act as a sarcomeric-like system to generate the force necessary to split transport carriers. Accordingly, there is an abnormal accumulation of uncoated vesicles close to cisternae after the knockdown of myosin II or the pharmacological blockade of its motor activity.<sup>176,180</sup> Many of them remain attached to cisternae, which argues in favor of the participation of myosin II in the fission process.<sup>179</sup> However, in another line of evidence, myosin II has been reported to be required only for motion but not for the biogenesis of PKD-dependent transport carriers at the TGN.<sup>181</sup>

With regard to myosin V, it has been recently reported that myosin Va interacts with only a subset of the Rabs associated with the endocytic recycling and post-Golgi secretory systems.<sup>182</sup>

Myosin VI is another myosin motor located in the Golgi.<sup>183,184</sup> It is a processive myosin that moves to the fast-depolymerizing minus-end pole of the microfilament. Myosin VI is involved, among many others in the maintenance of Golgi morphology.<sup>184,185</sup> The interaction between myosin VI and optineurin, a partner of Rab8,<sup>185</sup> acts at the TGN of polarized epithelial cells in the protein sorting and basolateral transport mediated by the clathrin adaptor-protein complex AP-1B.<sup>186</sup> Therefore, the known role of some Rab proteins as linkers of endocytic membranes to cytoskeletal motors is now also extended to the Golgi.<sup>187</sup>

Unconventional myosin 18A has also been located in distal Golgi membranes, where it binds to the PI4P-binding protein GOLPH3. It has been suggested that the GOLPH3–Myosin18 interaction couples actin filaments to Golgi membranes, and the tension generated by this interaction facilitates the maintenance of the extended Golgi-ribbon organization and flattens Golgi cisternae. In addition, it also seems to support secretory function, because the depletion of GOLPH3 blocks the exit of VSV-G from the TGN.<sup>100</sup> The contribution of this unconventional myosin to Golgi-associated membrane trafficking requires further characterization, because

it exhibits low motor activity.<sup>188</sup> Nonetheless, it has been recently reported that GOPPH3 links deoxyribonucleic acid (DNA) damage with Golgi fragmentation and dispersion,<sup>189</sup> revealing the DNA-PK-GOLPH3-MYO18A pathway to be necessary for cell survival upon DNA damage.

It is reasonable to think that as occurs with molecular forces (represented by actin motors), physical ones could also contribute to the shape, organization, and function of endomembranes in general and on the Golgi in particular. Whereas great progress has been made in addressing the contribution of membrane tension and curvature to coat-induced budding and molecular sorting and to membrane fission of transport carriers using *in vitro* systems,<sup>190</sup> the *in vivo* approach has remained elusive, mainly for technical reasons. However, a recent study in which optical tweezers were combined with confocal microscopy in living cells revealed that Golgi membranes are flexible and mechanically coupled, that actin confers rigidity to the GA, and that a mechanical constraint produces a switch from vesicular to tubular trafficking, linking forces with membrane fission.<sup>191</sup> The diagram shown in Figure 3 summarizes the variety of molecular processes in which actin or actin-based machinery has been reported at the Golgi in mammalian cells.

## The Golgi apparatus–actin interaction in other cellular systems

### Plant cells

Stationary actin filaments or actin bundles are the most prominent cytoskeleton element in plant cells. Attached to the actin bundles are the ER, vesicles, and numerous discrete clustered Golgi stack–TGN units, also known as Golgi bodies. They are highly variable in number (from a few tens to hundreds), depending on the plant-cell type and its developmental stage.<sup>7,10</sup> In polarized root hairs and pollen tubes, the TGN is segregated from Golgi bodies, which localize to growing tips, where together with actin, Rho/Rac members (ROPs and Rac1, respectively), Rab (Rab4a and Rab11), and ARF (ARF1) small GTPases regulate secretory and endocytic trafficking.<sup>192</sup> Also in this cell type, the motility and positioning of Golgi bodies is highly dependent on the actin organization, being faster and directional in areas containing actin-filament bundles and slower and nondirectional in areas with fine filamentous actin.<sup>193</sup>

In plants, most of the endomembrane compartments are in constant movement together with the cytoplasmic streaming whereby cellular metabolites are distributed throughout the cell.<sup>194</sup> Golgi bodies show actin-dependent dispersal and spatial organization.<sup>195,196</sup> They contain a fine fibrillar

material that is enriched in actin and spectrin- and myosin-like proteins.<sup>197,198</sup> The depolymerization of microfilaments with actin toxins uncouples the association between specific regions of the cortical ER with individual Golgi bodies;<sup>195,196</sup> however, and in contrast to animal cells,<sup>96</sup> it does not perturb the brefeldin A (BFA)-induced Golgi disassembly.<sup>199</sup> Therefore, cytochalasin or latrunculin treatments induce the aggregation of Golgi bodies and variably alter the Golgi morphology depending on the cell type and the period of treatment.<sup>198,200</sup> Actin toxins also perturb the coordinated movement of Golgi bodies and ER tubules.<sup>11,201</sup> Actin does not participate in ER–Golgi interface protein transport,<sup>202</sup> but it does contribute to post-Golgi trafficking to the plasma membrane and the vacuole. In the tip of growing cells like pollen tubes, actin filaments are the tracks through which Golgi-derived secretory vesicles are transported.<sup>203,204</sup> An intact actin–myosin system is required for the transport of cargo containing polysaccharides and the enzymes necessary for cell-wall morphogenesis, and the local differences in the actin–cytoskeleton organization determine where their secretion is required.<sup>89,205–207</sup> Golgi bodies are also propelled by plant myosin-family members, particularly the myosin XI class.<sup>208</sup>

### Yeast

Most components of the secretory pathway and many of the actin-based cytoskeleton are conserved between yeast and mammalian cells.<sup>209,210</sup> The actin cytoskeleton in yeast consists primarily of cortical patches and cables.<sup>211–213</sup> Many actin mutants accumulate large secretory vesicles and exhibit phenotypes consistent with defects in polarized growth,<sup>214,215</sup> which together with the polarized organization of actin cytoskeleton has suggested a role for actin in the positioning and orientation of the secretory pathway and polarized transport of late secretory vesicles to the plasma membrane.<sup>210,216</sup> A mutation of GRD20, a protein involved in sorting in the TGN–endosomal system, showed defects in the polarization of the actin cytoskeleton and impaired secretion of vacuolar hydrolase carboxypeptidase Y (but not other TGN-membrane proteins).<sup>216</sup> The actin-severing protein cofilin and Pmr1, the yeast ortholog of the secretory pathway calcium ATPase 1 (SPCA1), are both required for sorting at the late Golgi compartment.<sup>123</sup> Overexpression of Avl9p produces vesicle accumulation and a post-Golgi defect in secretion, and its depletion in a strain that also lacks Vps1 (dynamin) and Apl2 (adaptor-protein complex 1) impaired actin–cytoskeleton organization and caused defects in polarized secretion.<sup>217</sup> Concentration of late (but not early) Golgi elements at the

yeast bud (the site of polarized growth) depends on actin, which is transported along actin cables by yeast myosin V (Myo2).<sup>218</sup> Crucial in this process is Ypt11, a Rab GTPase that interacts with Myo2 and Ret2, a subunit of the coatamer complex. The polarization of late Golgi cisternae in the bud is not produced in the Ypt11Δ mutant.<sup>219</sup> The Rab protein Ypt31/32 present at the TGN directly interacts with Myo2 and the secretory vesicle Rab Sec4, whose interaction is modulated by PI4P levels.<sup>220</sup> Moreover, the Ypt31/32-Myo2-Sec4 complex interacts with the exocyst subunit Sec15, regulating post-Golgi trafficking and cell growth.<sup>221</sup>

In the early secretory pathway, actin depolymerization with actin toxins does not affect ER-to-Golgi protein transport,<sup>222</sup> but it does affect retrograde Golgi-to-ER trafficking, which is regulated by the ubiquitin ligase Rsp5, a protein that forms a complex containing COPI subunits and has as substrates the actin-cytoskeleton proteins Sla1, Lsb1, and Lsb2, which bind to the Arp2/3 activator Las17.<sup>223,224</sup>

### *Drosophila*

The *Drosophila* cellular model is an alternative to yeast to study the Golgi, because it shares many structural and functional similarities with the mammalian model, but importantly most *Drosophila* cells lack the characteristic mammalian Golgi ribbon. Instead, they present a scattered and fairly constant number of what are known as ER–Golgi units, which are ultrastructurally constituted by a pair of Golgi stacks.<sup>225</sup> The integrity of the actin cytoskeleton is crucial for Golgi-stack pairs, since actin depolymerization causes their splitting and perturbs Golgi inheritance, which requires duplication to form the paired structure. Abi and Scar/WAVE (but not WASP) are necessary in this process.<sup>226</sup> The inactivation of the golgin-like MT/actin-binding protein lava lamp prevents the necessary Golgi dispersal in the cellularization process.<sup>227,228</sup> *Drosophila* S2 cells showed that the depletion of the *tsr* gene (which codifies for destrin, also known as ADF/cofilin) aggregated and dilated Golgi membranes, inhibiting HRP secretion.<sup>229</sup> The mutant coronin proteins dpdo1 and coro, which regulate the actin cytoskeleton and govern biosynthetic and endocytic vesicular trafficking, severely perturbed embryonic development due to abnormal cell division and aberrant formation of essential morphogen gradients.<sup>230</sup>

### *Dictyostelium discoideum*

Cells of this social amoeba contain various types of vacuole, ER, and small Golgi stacks.<sup>231</sup> Comitín (p24) is a dimeric *Dictyostelium* actin-binding protein present in the Golgi

and vesicles that binds Golgi-derived vesicles to the actin filaments via the cytoplasmic exposed mannosylated glycans.<sup>232,233</sup> Villidin is another actin-binding protein that associates with secretory vesicles and Golgi membranes.<sup>234</sup> The centrosomal protein LIS1 (DdLIS1) links MTs, the nucleus, and the centrosome, and indirectly controls Golgi morphology. Mutants of this protein lead to MT disruption, Golgi fragmentation, and actin depolymerization.<sup>235</sup> AmpA is a secreted protein necessary for cell migration in an environment-dependent manner that also participates in the regulation of actin polymerization. It is found in the Golgi, but transported to the plasma membrane, where it regulates endocytosis.<sup>236</sup> In addition to Rho GTPases, *Dictyostelium* also contains other Rho-regulated signaling components, such as Rho nucleotide-dissociation inhibitors, the Arp2/3 complex, PAK, WASP, Scar/WAVE, formins, GEFs, and GAPs.<sup>237</sup> The acquisition of cell polarity during chemotaxis needs WASP, which localizes on vesicles whose formation in the Golgi requires the interaction between WASP with the pombe Cdc15 homology (PCH)-family protein members Nwk/Bzz1-p-like and syndapin-like proteins.<sup>238</sup> RacH is a closer protein to Rac and Cdc42 that localizes to compartments of the secretory pathway (nuclear envelope, the ER, and the Golgi) where it stimulates actin polymerization, and it also seems to be involved in actin-based trafficking of vesicles, but in contrast to AmpA, it is uncoupled from chemotaxis.<sup>239</sup>

## Conclusion

The positioning, architecture, and trafficking of the GA, which varies greatly in shape and number from one organism to another, is highly dependent on the cytoskeleton, particularly MTs and actin filaments. The cytoskeleton imposes the localization of the GA and determines its structure and function in terms of cargo segregation and transport-carrier formation. In most mammalian cells, the GA is organized in a ribbon-like shape and localized around the centrosome in the cell center. The centrosome is the major MT-organizing center in animal cells. It is responsible for organizing a radial MT network during interphase and the mitotic spindle during cell division. Recently, MT-nucleating activity has also been found to be associated with the GA. Both centrosome- and Golgi-nucleated MTs participate in ensuring the structural integrity of the Golgi ribbon and its pericentrosomal position, which could be relevant in processes that involve Golgi reformation, such as cell division. Translocation of transport carriers from the ER to the GA (anterograde transport) and from the GA to the ER (retrograde transport) also depends on

MTs and involves MT motors of opposite polarity, ie, dynein and kinesins, respectively. In addition to molecular motors, the Golgi-associated molecular machinery involved in the motion of transport carriers and nucleation of MTs includes Rab proteins (Rab6), coiled-coil proteins (golgin-160 and GMAP210), MT-stabilizing proteins (CLASPs), and centrosome-Golgi-associated proteins (AKAP450). On the other hand, in animal cells, the actin-dependent cytoskeleton is highly relevant in early events in the formation of Golgi-derived transport carriers, such as sorting and membrane fission. Actin is also necessary to maintain the characteristic flattened shape of cisternae. A wide actin-associated molecular machinery is documented, such as actin nucleators (ARP2/3 and formin-family members), actin regulators, effectors and binding proteins (RhoA, Rac1, Cdc42, N-WASP, and cortactin, among others), and actin motors (myosins I, II, VI, and 18). Altogether, this causes a fine regulation of the formation of retrograde and post-Golgi transport carriers, in which mechanical forces associated with local actin polymerization play a relevant role. In summary, MTs and actin filaments act in an almost indissoluble and coordinated manner in the architecture and function of the GA in many cellular systems.

## Acknowledgments

We are grateful to our respective past and present lab members for valuable discussions and support, and RMR particularly to M Arjona and C Marcozzi. Research in GE's and RMR's labs is supported by grants from the Ministerio de Economía y Competitividad (BFU2009-07186 and BFU2012-33932 to GE, and BFU2012-36717 and CSD2009-00016 to RMR). RMR's lab is also supported by the Consejo Superior de Investigaciones Científicas and the Junta de Andalucía (Spain).

## Disclosure

The authors report no conflicts of interest in this work.

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