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Connecting the Cytoskeleton to the Endoplasmic Reticulum and Golgi

Review

Pinar S. Gurel, Anna L. Hatch, and Henry N. Higgs*

A tendency in cell biology is to divide and conquer. For example, decades of painstaking work have led to an understanding of endoplasmic reticulum (ER) and Golgi structure, dynamics, and transport. In parallel, cytoskeletal researchers have revealed a fantastic diversity of structure and cellular function in both actin and microtubules. Increasingly, these areas overlap, necessitating an understanding of both organelle and cytoskeletal biology. This review addresses connections between the actin/microtubule cytoskeletons and organelles in animal cells, focusing on three key areas: ER structure and function; ER-to-Golgi transport; and Golgi structure and function. Making these connections has been challenging for several reasons: the small sizes and dynamic characteristics of some components; the fact that organelle-specific cytoskeletal elements can easily be obscured by more abundant cytoskeletal structures; and the difficulties in imaging membranes and cytoskeleton simultaneously, especially at the ultrastructural level. One major concept is that the cytoskeleton is frequently used to generate force for membrane movement, with two potential consequences: translocation of the organelle, or deformation of the organelle membrane. While initially discussing issues common to metazoan cells in general, we subsequently highlight specific features of neurons, since these highly polarized cells present unique challenges for organellar distribution and dynamics.

Introduction

In some respects, cell biological research resembles a group of people putting together a very large jigsaw puzzle. The tried-and-true method for jigsaw puzzle solving is 'divide and conquer'. For example, if the puzzle represents a horse in a pasture, one person might start with a bit of the horse's head, while another chips away at a leg (not necessarily knowing which leg), and a third might assemble some pasture. As each expands their sphere, they eventually make connections. At that point, the process moves rather quickly, with unanticipated connections.

Cell biology has, to some degree, cleared the first part of the process, reaching a point where connections are regularly made between previously separate fields. Sometimes, though, it is all too easy to stay close to the horse's head, avoiding linking up with the leg or the pasture. In this review, we consider connections between cytoskeleton and organelles (Figure 1), focusing on the involvement of actin and microtubules with the endoplasmic reticulum (ER) and Golgi. We remain limited puzzle-solvers in several ways: ignoring other cytoskeletal elements, such as intermediate filaments and septins; focusing on metazoans; and paying particular attention to one cell type, neurons.

We come from the cytoskeletal field, and as such may commit some sins of omission in terms of background. For detailed background, please refer to the following publications: actin dynamics [1]; actin nucleation by Arp2/3 complex, formins, or other proteins [2]; myosin motors [3]; microtubules [4,5]; and microtubule motors [6,7]. Here we briefly provide some salient features of actin and microtubules and largely direct readers to the above references for more details.

Actin filaments are two-stranded helical polymers of the 43 kDa actin monomer, and measure 7 nm in diameter. Filaments are polar, with 'plus' and 'minus' ends (which, for historical reasons, are more often called 'barbed' and 'pointed' ends). In non-muscle cells, all known filament growth occurs at the barbed end. In addition, the barbed end tends to abut a membrane surface, with the pointed end away from the membrane. Cytoplasmic actin concentration ranges from 50 to 200 μM in metazoan culture cells, with 50–70% polymerized at interphase ([8], F. Li, S. Nicholson-Dykstra and H.N.H., our unpublished results).

A major control point in actin filament growth is nucleation of a new filament, and three classes of 'nucleation factors' are known. Arp2/3 complex nucleates 'branched filaments' (Figure 2). While there is only one Arp2/3 complex, functional diversity is provided by its activators, such as WASP, N-WASP, Scar/WAVE proteins, WASH, and WHAMM, which are regulated by distinct mechanisms. A second class of actin nucleators is the formin proteins, which remain at the barbed end after nucleation and subsequently control filament elongation. In fact, in some cases formins might serve chiefly as elongation factors for filaments nucleated by Arp2/3 complex or other proteins. There are multiple formins (15 in mammals, 6 in *Drosophila* and 7 in *Caenorhabditis elegans*), providing the potential for diverse cellular roles. Finally, compound WH2 domain proteins (COWs) represent a third class of nucleator and include proteins such as Spire and Cordon Bleu. COWs can also synergize with formin proteins in actin assembly [9].

Actin filaments assemble in many places for many purposes. We count more than 20 known actin-based structures and there are certainly more remaining to be discovered [10]. While some actin-based structures, such as stress fibers, appear to be large and stable, the filaments in these structures are typically <1 μm long and turn over on a time scale of minutes. Other cellular actin filaments are even shorter (<200 nm) and turn over faster, the clearest examples being the Arp2/3 complex-assembled 'dendritic' networks at the leading edge of motile cells, around endosomes and at phagocytic cups. Structures assembled by formins or COWs are less well characterized in metazoan cells, but many are likely to be short and transient, such as those at mitochondrial fission sites [11]. We raise this point because short/transient actin filaments can be very difficult to identify by fluorescence or electron microscopy (EM), and this has hindered elucidation of their roles in the secretory pathway (as discussed in the Golgi section below).

Microtubules are hollow tubes with a 24 nm diameter — much larger than an actin filament. To illustrate the size difference between actin filaments and microtubules, two actin filaments could fit within the lumen of a microtubule. In most cellular circumstances, the tube is formed by 13 linear protofilaments that make lateral contacts. The building blocks of

Department of Biochemistry, Geisel School of Medicine at Dartmouth, Hanover NH 03755, USA.
*E-mail: henry.higgs@dartmouth.edu

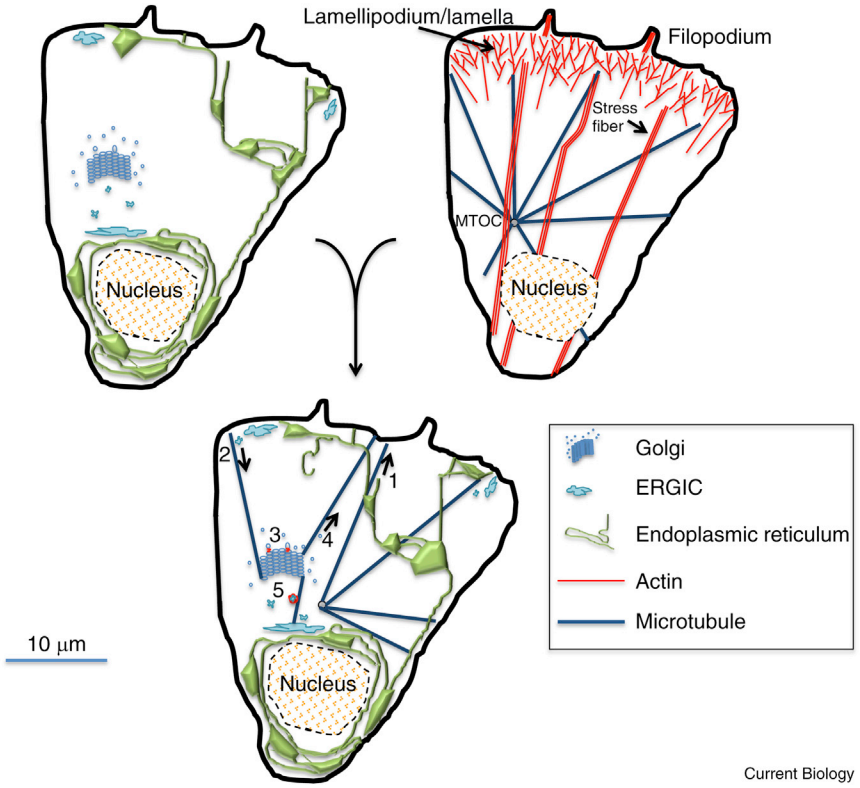

Figure 1. A cellular jigsaw puzzle of organelles and cytoskeleton.

Simplified representations of a 'generic' metazoan cell. The upper left cell depicts three organelles: ER (green), Golgi (blue). The ER-Golgi intermediate compartment (ERGIC), both central and peripheral, is shown in light blue. The upper right cell depicts abundant cytoskeletal structures: lamellipodium/lamella, filopodium and stress fibers for actin (red); and a microtubule array (blue) with its origin at the microtubule-organizing center (MTOC). The lower cell depicts known or postulated interactions between these organelles and cytoskeletal elements, including: 1) translocation of ER toward microtubule plus ends; 2) a set of microtubules originating at the *cis*-Golgi and being used for dynein-based transport from the ERGIC to the *cis*-Golgi; 3) actin involvement in membrane budding from the *trans*-Golgi network (TGN); 4) a set of microtubules originating at the TGN and being used for kinesin-based transport from the TGN to the periphery; and 5) actin involvement in ER-to-Golgi movement of vesicles/tubules.

these protofilaments are heterodimers of α- and β-tubulin (both about 50 kDa). Like actin, microtubules are polar, with a plus and a minus end. Both ends undergo dynamics (i.e. growth and shrinkage) [12], although the plus end gets most attention. In fibroblasts, about 70% of the approximately 90 μM cytoplasmic tubulin dimer is polymerized at interphase (based on [13,14] and our own calculations of cytoplasmic volume). While tubulin represents about 4% of cellular protein in many cultured cells, it reaches 25% in brain [14], partially due to the high density of microtubules in axons and dendrites. Cellular microtubules tend to be longer than actin filaments and largely originate at one place, the microtubule organizing center (MTOC) or centrosome, with minus ends remaining tightly embedded there. However, acentrosomal microtubules exist, the most relevant to this review being those originating from the Golgi [15,16].

An important function of the cytoskeleton is to provide the force required for membrane movement. This movement can result in membrane deformation (such as during yeast endocytosis or leading edge extension in cell motility) or membrane translocation from one point to another (such as GLUT4 vesicle translocation). Both actin and microtubules can generate force in two ways: by polymerization/depolymerization, or by serving as substrates for motor proteins (Figure 2). The actin-based myosin motor proteins all move in the barbed end direction, except for myosin VI, which is a pointed end motor. Myosin II is a special case, assembling into bipolar filaments that can exert contractile force. For microtubules, dyneins are minus-end-directed motors, while many kinesins are plus-end-directed motors. However, there are kinesin motors that move toward the plus end, and some kinesins are used for depolymerization or other functions. Of note, there are many members of the large myosin and kinesin families for which biochemical and/or cellular functions are unknown. Lastly, while motor activity is often associated with membrane translocation and cytoskeletal polymerization/depolymerization is associated with membrane deformation, motors certainly can affect deformation and polymerization/depolymerization can affect translocation.

While the importance of the cytoskeleton for membrane movement has long been appreciated, new connections are now being made with organelles not commonly thought to be associated with the cytoskeleton. A central theme in this review is the relationship between the function of actin/microtubules and either deformation or translocation of ER, Golgi, or transport intermediates.

Endoplasmic Reticulum

ER mediates a wide variety of cellular processes such as: synthesis, modification, quality control, and transport of proteins; Ca^{2+} homeostasis; and lipid synthesis/distribution. First described for its 'lace-like' reticular structure, this large organelle extends as a single membrane-bound entity to virtually all corners of the cell, and is composed of interconnecting sheets (also called cisternae) and tubules [17,18]. Tubules are approximately 50 nm diameter in mammals, and sheets are flattened double-membrane structures with approximately 50 nm lumenal space. Some ER sheets can be fenestrated in yeast and mammalian culture cells [19–21], with the latter measuring about 75 nm in diameter [19]. Sheets and tubules can interconvert in mammals [19,22,23].

ER tubules rely on several varieties of membrane-embedded proteins — including reticulons, DP1, and receptor expression enhancing proteins (REEPs) [24] — that stabilize the tightly curved membrane structure. New tubule branches can arise from the sides of existing tubules [25] and fuse with other tubules through the action of the dynamin family GTPase atlastin [26]. In sheets, several transmembrane proteins, such as Climp63, p180 and kinectin, are enriched in the flat region, while curvature-stabilizing proteins

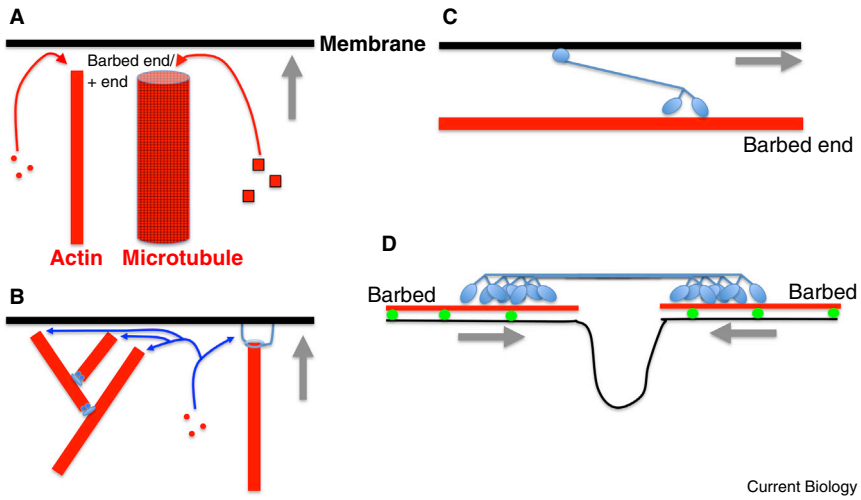

Figure 2. Mechanisms for cytoskeleton-based force generation on membranes.

(A,B) Polymerization-based force generation. (A) Membrane can be pushed by a polymerizing actin filament or microtubule via the addition of new polymer subunits to the plus (+) end (or barbed end for actin), which abuts the membrane. (B) Specific examples of actin polymerization-based force production: left, Arp2/3 complex assembling a branched filament network (Arp2/3 complex is at the branches); and right, a formin protein attached to the filament barbed end and to the membrane. Not shown are proteins containing multiple WH2 domains (e.g. Spire and Cordon Bleu), which can nucleate new actin filaments, possibly remaining at barbed ends in some cases. (C,D) Motor-based force generation. (C) Motor-based translocation along a filament: dimeric myosin motor moving towards the actin filament barbed end using its motor head groups, and attached to a membrane through a tail motif. One myosin (myosin VI) moves toward the opposite end of the actin filament (the pointed end). Motors that translocate on microtubules include kinesins (toward plus ends) and dyneins (toward minus ends). (D) Motor-based contraction: a non-muscle myosin II mini-filament, in which the motor heads are bound to two different actin filaments and move towards their respective barbed ends. In the process, myosin II causes constriction of the membrane attached to the actin filaments. Membrane, black; cytoskeletal elements, red; cytoskeletal-interacting proteins, blue. Gray arrows denote direction of membrane movement. Green dots denote proteins tethering actin filaments to the membrane. Formins bound to the barbed end could serve this purpose.

are excluded from these regions and instead enrich at the tightly curved sheet edges [27]. Suppression or deletion of curvature-stabilizing proteins causes reduction or abolition of tubules, whereas suppression of sheet-enriched proteins does not abolish sheets. These results might be explained by the fact that the tightly curved tubule is a high-energy structure compared with the flat sheet, which might be the default state. The regular spacing of membranes in the sheet does require stabilization, however, and interaction between the lumenal domains of Climp63 appears to serve this purpose in mammals [27]. Other specialized regions of ER include close connections with mitochondria, plasma membrane, Golgi, endosomes, and peroxisomes [28]. Mitochondria–ER contacts facilitate the exchange of molecules such as calcium and lipids, and have roles in stress responses and mitochondrial fission, with some of these functions perhaps being interrelated.

The structural heterogeneity of ER clearly contributes to its functional compartmentalization, but it may be going too far to say there is absolute delineation of function to either sheets or tubules. For example, sheets are often equated with ‘rough ER’ (containing translocon proteins and binding ribosomes), partially due to the abundance of sheet structure in ‘professional’ protein secretory cells, such as pancreatic beta cells or activated B lymphocytes. In addition, ribosome detachment might lead to an increase in tubules [19,23] and Climp63 appears to immobilize translocon complexes [29], suggesting a reciprocal relationship between sheets and translation/translocation. However, ER tubules can also bind ribosomes in yeast [21] and contain translocon proteins in mammals [27], albeit at lower levels than in sheets. Further work is required to determine how much translation/translocation actually takes place on tubules. The ER stress response might also be enhanced in sheets, since cells appear to increase the sheet:tubule ratio when under stress, perhaps to increase lumenal volume to handle increased levels of unfolded protein [20,21]. It is unclear what specific functions might be better conducted by tubules, but their transport on microtubule tips might suggest a role in Ca^{2+} dynamics (see below). Also, the association between ER and mitochondrial fission could be specific to ER tubules from the examples shown [11,30], although this has not been formally tested.

ER–Cytoskeleton Interactions

Although ER can form a reticular network independently of cytoskeletal structures [31], ER distribution and sheet/tubule balance are influenced by microtubules in mammalian cells [32–36]. There are two types of microtubule-dependent ER movement: sliding, involving motor-based transport along pre-existing stable acetylated microtubules; and movement mediated by the tip attachment complex (TAC), whereby a plus end-attached ER tubule extends with the growing microtubule [35,37] (Figure 3). While motor-based transport represents the prevalent mechanism in the cells studied so far, more details are available for TAC-mediated movement, although there are controversies regarding the mechanism involved and the cellular function of such movement.

In fact, there is the exciting possibility that TAC is directly involved in one of the major functions of ER — a store of Ca^{2+} ions that can be discharged upon appropriate stimulation. The ER lumen contains 10,000-fold higher Ca^{2+} concentrations than the cytosol [38] and must be replenished efficiently. One source of Ca^{2+} for the ER is the extracellular milieu, and ER tubules contact the plasma membrane for replenishment in a TAC-dependent manner through a process called store operated calcium entry (SOCE) [39,40]. The interacting proteins relevant for TAC motility are EB1, bound to the microtubule plus end, and STIM1, an ER transmembrane Ca^{2+}-binding protein that serves as a lumenal Ca^{2+} sensor [37,41,42]. Upon depletion of ER Ca^{2+} stores, STIM1 aggregates and relocalizes to ER–plasma membrane junctions where it interacts with Ca^{2+} release activated calcium (CRAC) channels to promote influx of extracellular Ca^{2+} into the ER [41,43,44].

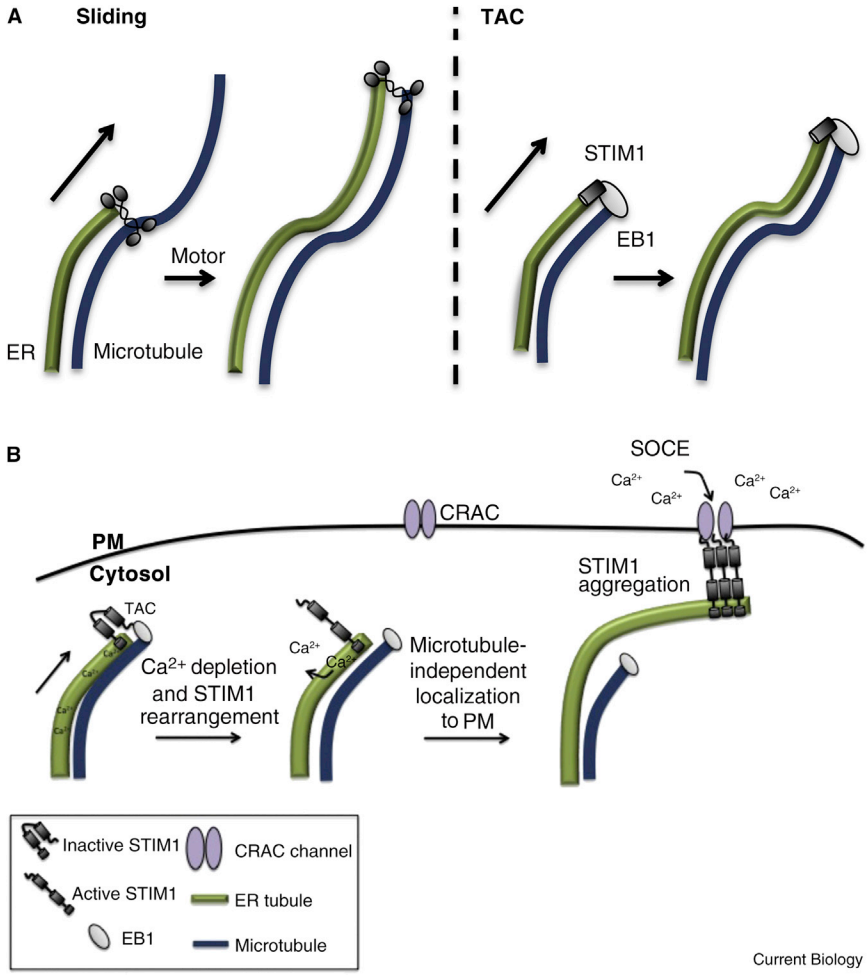

Figure 3. Microtubule-based ER motility.

(A) Two mechanisms of microtubule-based ER translocation. 'Sliding' refers to kinesin-mediated ER movement along an existing microtubule. 'TAC' (or tip attachment complex) mediated motility refers to ER moving with the elongating plus end of a microtubule, through interaction between STIM1 on the ER and EB1 on the microtubule plus end. (B) TAC and ER calcium dynamics. In addition to binding EB1, STIM1 is an ER Ca^{2+} sensor through its luminal EF hand motifs. When Ca^{2+} levels in the ER are low, STIM1 interacts with plasma membrane calcium channels (CRAC channels) to mediate store operated calcium entry (SOCE). One mechanistic model postulates that TAC is necessary to position STIM1-containing ER near appropriate sites for SOCE. Upon depletion of ER Ca^{2+}, STIM1 aggregates, dissociates from the microtubule plus end, and engages the plasma membrane (PM) Ca^{2+} channel.

The precise role of TAC-based ER motility in SOCE is still debated and appears variable among cell types [41,44–46]. Early theories postulated that, upon Ca^{2+} depletion, TAC is activated as an initial step in SOCE. However, STIM1 appears to dissociate from EB1 upon its Ca^{2+}-mediated aggregation [41]. A current model suggests that TAC-mediated ER movement is required prior to SOCE to position STIM1 throughout the ER, with ER Ca^{2+} depletion causing microtubule-independent STIM1 translocation to the plasma membrane [46]. Interestingly, this process appears important in the pathogenesis of *Clostridium difficile*, which appears to hijack the host cell ER through re-routing of STIM1-mediated TAC-based motility [47].

The molecules mediating and regulating sliding ER transport are less well understood. While kinesin-based plus-end transport to the cell periphery appears to occur [35,37], the specific kinesin motor is not known. The sheet-enriched protein kinectin is known to bind kinesin [48]. While overexpression of kinectin's kinesin-binding domain perturbs ER dynamics [49], examining kinesin-mediated ER dynamics in kinectin-suppressed cells would be a more satisfying way to rule out indirect effects. There is clear evidence that ER can associate with dynein and move toward microtubule minus ends in *Xenopus* extracts [50,51] and mammalian cells [52].

A number of other ER proteins have also been shown to bind microtubules, including Climp63, p180, and certain members of the REEP family (Figure 4), although the exact role of microtubule binding is unclear in all cases. The short cytoplasmic amino-terminal region of Climp63 binds microtubules directly [53], and the Climp63–microtubule interaction appears to decrease the diffusion rate of the translocon complex [29]. A region of the extensive carboxy-terminal cytoplasmic region of p180 binds and bundles microtubules, perhaps dependent on dimerization of this region [54], and overexpression results in increased microtubule acetylation. The function of p180 appears to be in the translation-independent localization of specific mRNAs to the ER membrane [55]. One member of the REEP protein family, REEP1, has been shown to bind microtubules directly through its carboxy-terminal region, and sequence homology along with cellular experiments suggest that REEP1–4, but not REEP5 and REEP6, bind microtubules [56]. Finally, a specific isoform of spastin, termed M1 spastin, is associated with the ER through an amino-terminal hairpin region that also confers atlastin binding [56]. Spastin contains a carboxy-terminal hexameric AAA ATPase domain that has microtubule-severing activity as well as a second microtubule-interacting region between the hairpin and the AAA ATPase domain [57].

Mysteriously, microtubules appear to play an important role in the balance between ER sheets and tubules, since microtubule depolymerization causes an impressive accumulation of sheets within minutes of treatment [32,36]. Given the many microtubule-associated ER proteins in both sheets (Climp63, p180, and kinectin) and tubules (specific REEPs and spastin M1), the mechanism behind this transition is unclear. For example, it would be interesting to know how Climp63 phosphorylation, which apparently inhibits microtubule binding [58], influences sheet/tubule balance. Conversely, REEP1 and the spastin M1 variant have curvature-stabilizing domains that should drive tubule assembly, so how does microtubule binding modulate their

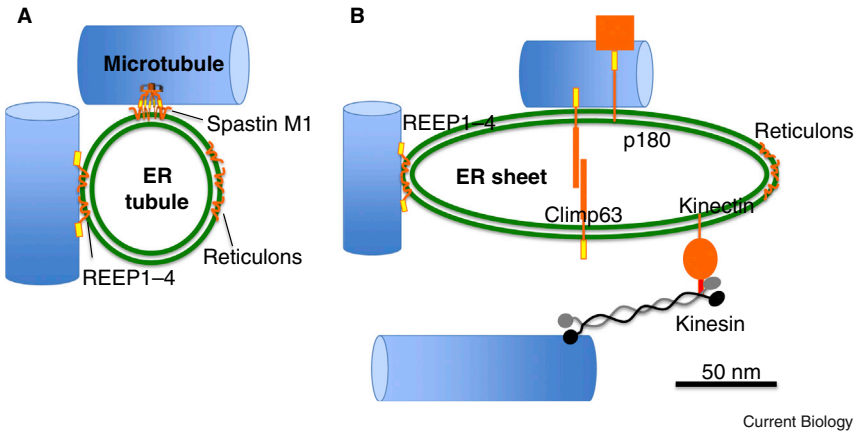

Figure 4. Known microtubule-binding proteins on ER tubules and sheets.

(A) ER tubule. The mammalian ER tubule is shaped by proteins that stabilize tight membrane curvature. These proteins contain one or two hydrophobic hairpin segments that can embed into the cytoplasmic leaflet. The reticulons have no known microtubule-binding capacity but some members of the structurally similar REEPs (REEP1–4) have a carboxy-terminal microtubule-interacting region. Spastin is a microtubule-severing protein containing a hexameric AAA ATPase domain (the severing domain) and a separate microtubule-binding region. In addition, the spastin M1 variant has an amino-terminal extension containing one hairpin sequence that can bind ER. (B) ER sheet. Curvature-stabilizing proteins are excluded from the flat region of the sheet by mechanisms that are poorly understood. Among the sheet-enriched proteins are Climp63, p180 and kinectin. Climp63 has an extensive lumenal region that interacts homotypically and is thought to serve as the 'spacer' that maintains a 50 nm lumenal width. P180 has an extensive cytoplasmic domain that contains a microtubule-binding domain and may act in translation-independent localization of specific mRNAs to the ER membrane. Kinectin's cytoplasmic region interacts with a region near the carboxyl terminus of kinesin. The diagrams are scaled to show relative diameters of ER (50 nm for both tubule and sheet) and microtubule (24 nm) and length of kinesin. The depictions of Climp63, p180 and kinectin show the approximate relative amounts within and without the ER lumen. ER membrane, green double line; proteins, orange; microtubule-binding region, yellow; kinesin-binding region, red; microtubule, blue.

localization? As a side note of no direct relevance here, it is interesting to us that both microtubules and ER tubules are tubes of somewhat similar sizes. At times we idly wonder about the hidden world that might exist within the microtubule lumen.

In contrast to plants and yeast [59,60], actin does not appear to play a central role in ER movement or morphology in generic metazoan culture cells, although some evidence suggests that actin and myosin act in retrograde ER transport [35,61] and that actin and ER associate via filamin proteins [62]. Interestingly, actin may play a role in the sheet-to-tubule transition through myosin Ic [63]. Also, actin functions in neuronal ER distribution into dendritic spines, as discussed below [64,65]. A prenylated isoform of the formin INF2 is tightly bound to ER but appears to play no clear role in ER dynamics [66]. Interestingly, however, ER-bound INF2 does play a role in mitochondrial fission [11], demonstrating the capacity of ER to influence other organelles.

Neurons — Challenges for ER Distribution and Function

Given the enormous length and miniscule width of neurons (dendrite diameter 2–5 μm, axon diameter <2 μm), it is a marvel that ER distributes as a continuous network from one end to the other, even entering dendritic spines [61,67]. Super-resolution fluorescence microscopy and EM tomography show the intricacy of ER in dendrites from hippocampal neurons both in culture and *in situ*, with multiple branched tubules often emanating from regions of higher complexity (which one might be tempted to call 'sheets') that correlate with areas of high dendritic spine density [68]. This structural complexity results in functional compartmentalization in the ER, by reducing diffusion of a subset of newly synthesized plasma membrane proteins prior to ER exit [68]. Mechanisms for inducing this compartmentalization are not fully understood, but microtubules clearly play a role through CLIMP63 [68,69].

Before discussing ER in neurons, we provide some general features of neuronal microtubules and actin (Figure 5). Both axons and dendrites are microtubule rich, with microtubules uniformly oriented in axons (plus ends distal) and of mixed orientation in dendrites [70]. Axonal microtubules do not run the entire length but are staggered along the axon [71,72]. A similar distribution is assumed in dendrites, but has not been observed directly to our knowledge. Many of these microtubules are acentrosomal (not emanating from the centrosome/MTOC), since the MTOC is in the cell body. Indeed, most microtubule nucleation in mature cultured hippocampal neurons is acentrosomal [73]. Although axons and dendrites contain much less actin than tubulin, actin does enrich at several places: axon initial segments, axonal termini and dendritic spines/filopodia. Interestingly, dendritic filopodia appear different from 'normal' filopodia, in that their actin filaments are not uniformly oriented and they contain myosin II [74,75]. Finally, a recent super-resolution fluorescence microscopy study identified two additional, low-abundance, actin-based structures: longitudinally running filaments in dendritic shafts; and regularly spaced (190 nm periodicity) spectrin–actin structures forming bands around axonal shafts [76].

Returning to ER, how does it get from the dendritic shaft into dendritic spines, where Ca^{2+} release from the ER is crucial for synaptic plasticity and memory [77]? Microtubules can enter spines [78–81], but spines also contain actin filaments. A recent publication demonstrates an elegant mechanism whereby myosin Va conducts short-range ER transport along actin filaments into the dendritic spine, with myosin Va localizing to the ER tip [82]. Thus, gross dendritic ER morphology might be microtubule dependent but local ER import into spines might be actin dependent. Given that ER enters these spines to release Ca^{2+}, and STIM1 is involved in both Ca^{2+} sensing and ER transport, it will be interesting to see whether STIM1 plays any role in ER dynamics here. Of note, manipulation of actin filaments in hippocampal neurons affects Ca^{2+} release from ER [83].

As opposed to dendrites, axons are considered to be largely devoid of secretory machinery [84,85] (although this might not be true for some peripheral neurons [86]). Nevertheless, axons do contain extensive smooth ER that tracks

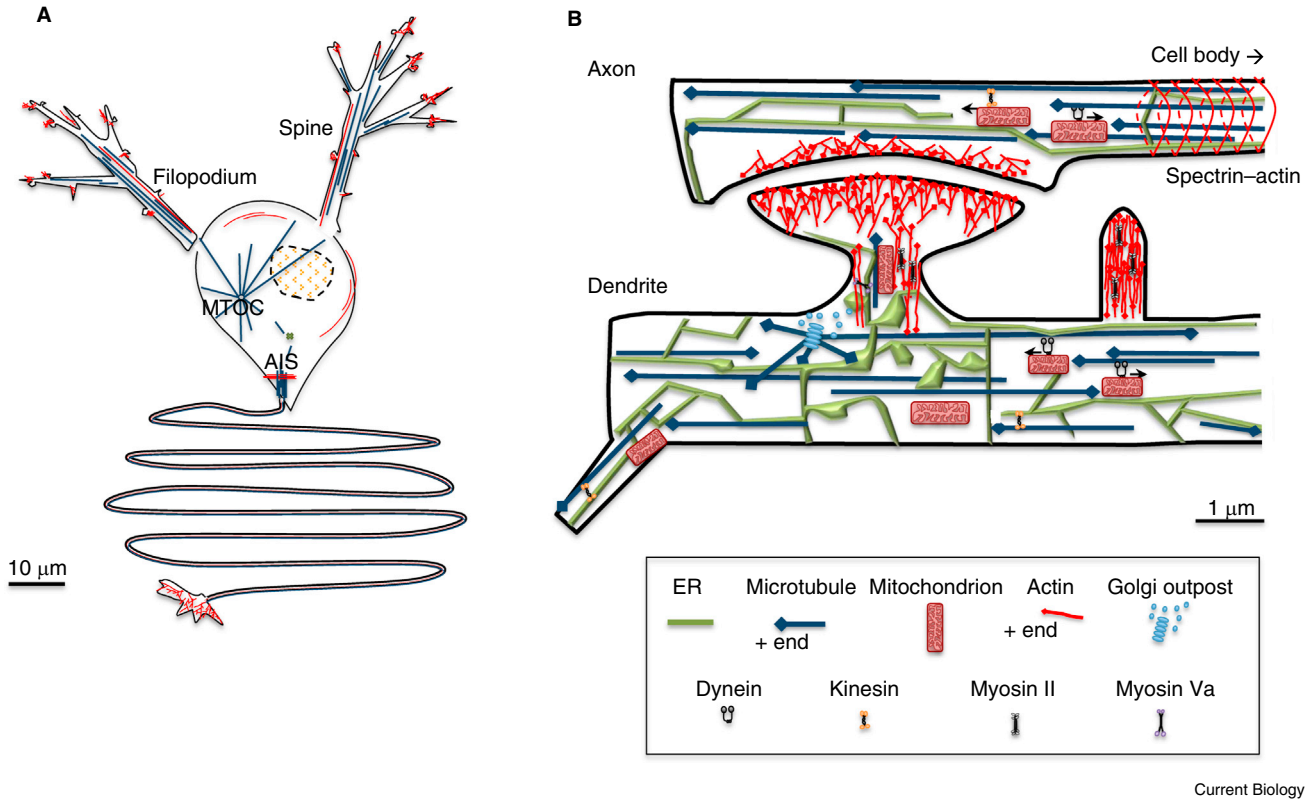

Figure 5. Cytoskeleton and organelles in neurons.

(A) Schematic representation of actin and microtubules in a 'generic' neuron, with one axon and two dendrites emanating below and above the cell body, respectively. The narrow axon (>2 μm diameter) is microtubule rich, with actin filaments most abundant in the axon initial segment (AIS) and the synapse/distal terminus. The dendrite is also narrow (<5 μm), but wider than the axon and tapers in width distally. As with axons, dendritic shafts contain abundant microtubules, with actin filaments enriching in dendritic filopodia and dendritic spines. Microtubules extend from the MTOC, but are also produced by katanin/spastin-mediated severing in the cell body. (B) Close-up of the cytoskeleton and organelles in the vicinity of a synapse. Microtubules (blue) are of uniform orientation (plus ends marked with diamonds) in axons, and mixed orientation in dendrites. A recent study shows that there is a small number of actin filaments running longitudinally in dendrites and a periodic spectrin–actin structure at 190 nm intervals in axons (only actin banding shown here). There are at least two populations of actin filaments in dendritic spines: an Arp2/3 complex-dependent branched filament network at the tip, and a set of anti-parallel filaments in the shaft. Myosin II also is present in the shaft, possibly making these structures contractile. A similar actin and myosin II arrangement exists in shafts of dendritic filopodia, but they are not bulbous at the tips. Both axons and dendrites contain ER. In dendrites, ER expands near dendritic spines, developing sheet-like structures and becoming highly branched. ER can move into the spine along actin filaments through myosin Va. Golgi is considered to be absent from axons and rare in dendrites, but small 'Golgi outposts' are sometimes found near dendritic spines, and microtubules can originate at these outposts. Dynamic microtubules can enter the dendritic spine, but their origins are unknown. Mitochondria transit along microtubules in both axons and dendrites.

all the way to axon termini [61,84,87]. In peripheral neurons, this axonal ER consists of a large number of branched tubules running parallel to the axonal axis, with some of these tubules in close proximity to the plasma membrane and occasional elaboration into sheet structures [87] (C. Blackstone and M. Terasaki, personal communication). The importance of axonal microtubule–ER interactions in peripheral neurons is suggested by the disease hereditary spastic paraplegia (HSP), which affects axons in long peripheral neurons [56]. Two proteins mutated repeatedly in HSP are the microtubule-binding proteins REEP1 and spastin. The exact functional connection between spastin and ER structure/function in axons is unclear, although M1 spastin is highly enriched in spinal cord neurons, which are also compromised in HSP [88], and is abundant at axonal branch points in cultured neurons [89]. One proposed neuronal role of spastin is to sever microtubules in the cell body for export to axons, which might affect ER distribution indirectly [90,91]. However, spastin also co-localizes with REEP1 in axons [56] and spastin suppression causes neuronal defects, even though katanin, another microtubule severer, is still present [89].

Golgi

Discovered over 100 years ago [92], the Golgi has become no less beautiful with age. The basic structural unit is the cisterna, a flattened membrane of roughly 20–40 nm thickness and 500–1000 nm in the other two dimensions [93]. Cisternae associate vertically to form a Golgi stack. In mammals, the number of cisternae per stack varies roughly between four and eleven. The two outermost cisternae, the ER-facing *cis*-cisterna and the opposite-facing *trans*-Golgi network (TGN), are morphologically different from the more central (medial) cisternae, being more vesiculated (Figure 6). A revised nomenclature for Golgi cisternae, based more on function, has recently been proposed [94].

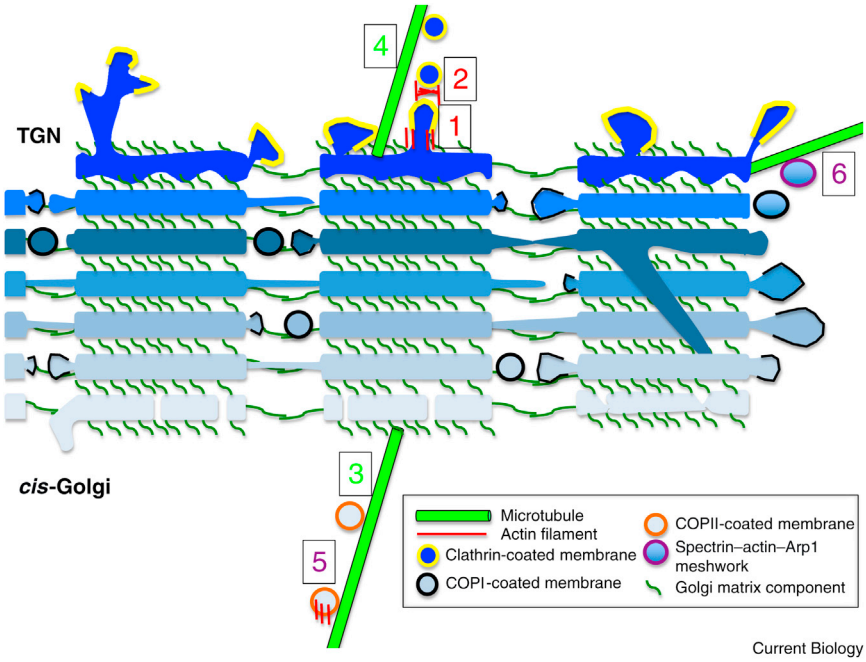

Figure 6. Possible roles for actin and microtubules in Golgi function.

Schematic cross-section of three laterally connected mini-stacks from mammalian Golgi*, with individual cisternae in blue (lightest are *cis* and darkest are TGN). Golgi matrix proteins (dark green) tether cisternae vertically within a mini-stack, and horizontally between mini-stacks. A subset of parallel cisternae establish lateral membrane connections, which are likely to be highly dynamic. Occasionally, intercisternal membrane connections might occur within a mini-stack (one depicted here). COPI-mediated vesicle transport (black banding around membrane) occurs from the edges of mini-stacks. COPII-coated vesicles arrive at *cis*-Golgi via the ERGIC. At the TGN, exiting transport membranes are generally clathrin coated (yellow banding). From our interpretation of the literature, we postulate six known or highly likely instances of cytoskeletal involvement in Golgi dynamics, some being actin dependent (red numbers), some microtubule dependent (green numbers) and some involving both actin and microtubules (purple numbers): (1) tubulation of TGN, involving actin, Arp2/3 complex and myosin 1b; (2) fission of transport vesicles from TGN, involving actin and myosin II; (3) transport of COPII vesicles to *cis*-Golgi-attached minus ends of microtubules by dynein; (4) transport of clathrin-coated vesicles away from TGN by kinesins; (5) transport of tubulovesicular membranes from the ERGIC to *cis*-Golgi, involving actin, microtubules and WHAMM; and (6) coating of Golgi-derived membranes with spectrin, possibly containing actin and/or Arp1, and interacting with microtubule motors in an as yet undefined manner. *Disclaimers: this is an 'artist's rendition' based on the available literature, and intended to provide food for thought. The membranous connecting regions between lateral cisternae could be very different from those depicted. The direction of movement of the COPI vesicles within the Golgi is kept intentionally vague, due to uncertainty in the field. We make no attempt to depict cisternal maturation, though there is good evidence for this. From EM studies, the Golgi matrix is likely to be much more dense than depicted here. The matrix is also of heterogeneous protein composition, with some proteins (e.g. GM130) at the *cis* face and other proteins (e.g. GCC185) at the TGN face. Finally, we do not depict the fenestrae that have been documented in cisternae.

In functional terms, protein cargo arrive at *cis*-Golgi as vesicles or tubulovesicular structures, undergo a series of modifications as they transit through the cisternae, then are packaged into clathrin-coated vesicles at the TGN for transport to multiple destinations, such as other organelles, the plasma membrane, or the extracellular environment, which requires cargo sorting. The TGN also receives vesicles delivered from some of these regions. Golgi-resident proteins are brought back to their correct region of the stack through coatomer I (COPI) vesicle trafficking [94,95].

The last two paragraphs paint a rather bucolic scene of harmony. In reality, Golgi structure and function are much more confusing and controversial [94–99]. Major questions regarding mammalian Golgi include: what is the mechanism of anterograde transport (through vesicles or through maturation/movement of an entire cisterna)? How do large cargos (bigger than can be handled by 50 nm vesicles) get transported? And how are different rates of protein transport accommodated in the same Golgi [94,100]?

In addition, metazoan Golgi structure is much more intricate than that depicted in most textbooks, with several well-documented but often overlooked features (Figure 6). First, metazoan Golgi generally consists of a group of 'mini-stacks' tethered in parallel to form a Golgi 'ribbon' [93,101], with a rough estimate of about 100 mini-stacks per Golgi ribbon in an interphase HeLa cell (A.D. Linstedt, personal communication). The mini-stacks are held together by lateral membrane fusion between a subset of adjacent cisternae, and these connections are likely to be highly dynamic [99]. We state 'likely' because these connections are <0.5 μm and are buried within the Golgi structure, thus are difficult to image dynamically. Second, small pores or 'fenestrae' exist in many cisternae, particularly in the connecting membranes between mini-stacks [93]. Third, tubular intercisternal connections are periodically present within individual Golgi stacks [102,103]. The precise significance of these structural features is unclear.

Another feature of metazoan Golgi is the matrix of proteins that coats each cisterna. This matrix is necessary for tethering transport vesicles as well as for maintenance of cisternal associations, both longitudinally within mini-stacks and laterally between mini-stacks [104]. Made up primarily of peripheral membrane proteins of the Golgi reassembly stacking protein (GRASP) and golgin families, the matrix is highly dynamic because these coiled-coil proteins associate and disassociate both with the Golgi and with each other, in part controlled by well-known kinase pathways [105]. The matrix is non-uniform, with some matrix proteins, such as GM130, associating with cisternae towards the *cis* face, and others, such as GCC185, associating with the TGN. Disruption of key matrix components causes Golgi fragmentation.

In the parallel universe of the cytoskeleton, findings have accumulated suggesting that both actin and microtubules play important roles in Golgi structure and function. For the most part, however, clear mechanistic links have not been made. The study of Golgi–cytoskeleton interactions poses challenges for at least two reasons. First, the Golgi itself is relatively small, and its component mini-stacks are even smaller. Second, the Golgi is often found near the nucleus

and the MTOC, and specific actin and microtubule interactions can easily be obscured by the abundance of both cytoskeletal elements in this region.

Before starting a more detailed discussion, we give a brief overview of Golgi–cytoskeleton connections. A general observation is that microtubule depolymerization causes Golgi fragmentation, whereas actin depolymerization causes Golgi compaction [106,107]. Microtubules are nucleated at the Golgi in a manner dependent on Golgi matrix proteins, both at the *cis*-Golgi and TGN, and maintain minus ends at these sites [15,16]. On the *cis* face, GM130 is thought to recruit γ-tubulin via an association with the γ-tubulin-interacting scaffold protein AKAP450. On the TGN face, GCC185 association with CLASP proteins is thought to stabilize growing microtubule seeds. Dynein drives at least some vesicle transport towards Golgi, while kinesins take vesicles away [6,108]. Inhibition of dynein or its cargo adaptor dynactin causes Golgi fragmentation [109].

There is evidence for diverse Golgi–actin connections as well. Three actin-polymerizing formin proteins have been linked to Golgi structure, with FMNL1γ and INF2 promoting Golgi compaction, and mDia1 promoting Golgi fragmentation [110–112]. Other actin-binding proteins influencing Golgi structure are WHAMM, cortactin, and myosin 18A [113–116]. Arp2/3 complex associates with Golgi membranes [117], and plays a role in transport [118]. Cofilin is associated with the TGN, and plays a role in cargo sorting [119,120]. A spectrin–actin meshwork is also thought to be present at or close to the Golgi, with its integrity affecting Golgi function [109,121,122]. Finally, multiple myosins (from the I, II, VI and XVIII families) play roles in Golgi structure and function [114,118,123]. So, how might this all fit together? (One topic we ignore at our peril is the structure/function of plant Golgi, which is better understood at many levels: as one example, most plant Golgi exists as individual mini-stacks that undergo rapid myosin-mediated transport along actin filaments [124].)

What Is 'Golgi-Associated Actin'?

The evidence for actin association with the Golgi is largely based on immunofluorescence localization of actin-binding proteins [110,118,119,123], and some have been shown to bind Golgi in cell-free assays [117]. Two issues should be raised here. First, these proteins are often abundant around Golgi, but are also found in vesicular structures surrounding the Golgi or elsewhere [116,122]. Sometimes, the proteins shown to affect Golgi structure have little or no actual enrichment there [111,112]. Second, the Golgi enrichment can be at one side of the stack, often towards the TGN [118,123].

What is the evidence for the presence of actin filaments themselves on Golgi? Standard actin staining using phalloidin does not conspicuously label Golgi in mammalian cells, but since the cytoplasm is often filament rich, one may miss relatively small accumulations. Closer examination by fluorescence microscopy reveals actin 'puncta' in the Golgi region [111,118,123]. These puncta are remarkably consistent in number (about 13 per Golgi in U2OS cells), decrease upon suppression of the formin INF2, and often coincide with myosin 1b and Arp2/3 complex enrichment [111,118]. High-magnification fluorescence images of these puncta suggest that they actually lie near the TGN membrane, not on it [118].

These actin puncta have not been obvious by EM, and actin is not mentioned in several cryo-EM tomography studies of Golgi [102,103,125]. However, there are major difficulties in EM imaging of both actin and membranes simultaneously, in that traditional fixation/contrast enhancement techniques for thin-section EM are damaging to actin filaments [126,127], whereas many techniques optimal for actin filaments either destroy membranes or provide little membrane contrast [128,129]. Cryo-EM tomography techniques are extremely promising, but there are still some caveats. First, some cryo-EM techniques still include potentially actin-damaging treatments after the freezing stage [93,102,103]. Preservation of large structures such as stress fibers under these conditions provides imperfect evidence of actin integrity, since many of the organelle-interacting actin filaments are likely to be short and labile. Second, short actin filaments might be difficult to detect by cryo-EM tomography even if well preserved, due to their orientation relative to the electron beam or to the fact that they might just be plain short [130,131]. As evidence for the difficulties in imaging short actin filaments by EM, there was a 15-year debate on the morphology of actin filaments at the leading edge of motile cells [132], and in this case we actually knew filaments were there!

The association of a specific spectrin (the βIII isoform) with Golgi has been known for some time [133,134], as has the Golgi association of specific forms of the spectrin-associated proteins ankyrin [135,136] and protein 4.1 [137]. Suppression of βIII spectrin disrupts Golgi structure [122]. However, the localization pattern of βIII spectrin is not confined to the Golgi, and appears rich on vesicular structures elsewhere in the cytoplasm [122,134]. This feature might suggest a role for spectrin more in transport to or from the Golgi rather than directly in Golgi morphology.

Another feature of βIII spectrin might draw it closer to microtubules than to actin. The classical spectrin–actin network from erythrocytes consists of a short (14 subunit) actin filament at the intersection of much larger spectrin oligomers [138]. Interestingly, βIII spectrin associates with the actin-related protein, Arp1 [139], which forms a short (8 subunit) filament as part of the dynactin complex, which links the dynein motor to cargo [140]. One possibility is that a Golgi-linked βIII spectrin–dynactin meshwork could interact with dynein, possibly contributing to selective cargo transport.

What Are 'Fragmented Golgi'?

What does the fragmented Golgi phenotype, induced by Golgi matrix disruption, microtubule depolymerization and other treatments [106,110,111], mean in terms of actual Golgi structure? In the case of microtubule depolymerization, the fragments are Golgi mini-stacks that localize near ER exit sites (ERES) and appear to be fully functional for transport [106]. Presumably, microtubule depolymerization disrupts interstack membrane connections within the ribbon, but it is unclear what the mechanism would be. It is also unclear how suppression of the formins FMNL1γ and INF2 might fragment Golgi, but this could possibly be related to the ability of formins to bind microtubules [141–143].

What is the dispersive force that drives fragmented Golgi to ERES? The fact that Golgi disperses upon microtubule depolymerization suggests that this is not a kinesin-driven process, although there could be stable microtubule tracks that remain. Given that actin depolymerization [107] and suppression of three actin-associated proteins — mDia1, cortactin and myosin 18A [112,114,115] — causes the opposite effect (Golgi compaction), actin has been suggested to

contribute in some way to Golgi fragmentation. There have been suggestions that myosin 18A activity on actin filaments might provide a tensile force to expand the Golgi [114], but an important caveat is that no actual motor activity has been detected for myosin 18A [144,145]. Interestingly, Golgi-associated Cdc42 has a negative effect on dynein-mediated Golgi transport and inhibits Golgi ribbon re-assembly after washout of the microtubule-depolymerization agent nocodazole in an actin-dependent manner [146]. This effect might occur through Arp2/3-mediated actin polymerization [117,147].

It is worth remembering that Golgi structure is highly sensitive to changes in membrane entry or removal [99]. Many of the effects mentioned above lead to changes in import and/or export, and thus could have indirect effects on Golgi structure [6,109]. For example, cortactin suppression causes impressive changes in Golgi morphology, but acts on late endosome/lysosome dynamics [115].

Possible Mechanisms for Actin Effects on Golgi

One interpretation of the current literature suggests that actin is not involved in the maintenance of Golgi structure but in transport carrier production, either at the budding stage or in fission from the Golgi surface. The clearest picture is at the TGN, where Arp2/3 complex, myosin I, myosin II, and cofilin have been shown to play roles [118–120,123,148]. The combination of Arp2/3 complex and myosin I might mediate membrane deformation of the nascent transport carrier, which may be more of a tubule than a transport vesicle [118]. Subsequently, myosin II-based contractility might serve in fission of these tubules from TGN [123]. Cofilin's function might be to accelerate actin depolymerization during these processes, although a link between cofilin and the Ca2+ pump SPCA1 seems to suggest additional roles in selective cargo trafficking [119].

It must be noted that cargo sorting is of paramount importance at the TGN, with at least three types of export: to endosomes/lysosomes; to the plasma membrane; and an additional pathway for glycosylphosphatidylinositol (GPI)-linked proteins [149]. Many of the proteins noted above, including Arp2/3 complex, myosin 1b and cofilin, seem to affect only subsets of transported proteins. Possibly, one regulated aspect of cargo sorting in this context may be transport carrier size and shape, a parameter that actin is well suited to influence through its ability to deform membranes.

Actin also appears to be involved in other phases of transport to and from Golgi. One example, discussed in a later section, is the role of the Arp2/3 complex activator WHAMM in transport from the ER-Golgi intermediate compartment (ERGIC) to the Golgi, where the ability to tubulate membranes might be especially useful in transport of larger cargo [113]. Another role for actin might be in COPI-mediated retrograde transport. Arp2/3 complex, and its activators N-WASP and Cdc42, can associate with Golgi through the Arf1-stimulated COPI coat [116,117,150].

We wonder about other possible functions for actin in Golgi structure and function, such as: dynamics of the tubular connections between mini-stacks in the Golgi ribbon; establishment of intercisternal connections within a stack; or dynamics/distribution of fenestrae within cisterna. These features can change along with changes in secretory activity [99,102,103], and almost certainly vary greatly with cell type. A shift from generic culture cells to primary cell systems might reveal possible connections more clearly.

Golgi in Neurons — Life at the Outpost

The above discussion brings us to neurons, where secretion poses particular issues. Small 'Golgi outposts' are present in dendrites, in addition to the main Golgi ribbon in the cell body. It is unclear what role the cytoskeleton might play in these specialized secretion systems, but the fact that dendritic Golgi outposts are microtubule nucleation sites [151] highlights the Golgi–microtubule link.

Mutations in βIII-spectrin can cause spinocerebellar ataxia type 5 (SCA5), a disease traceable in Abraham Lincoln's descendants and resulting in motor abnormalities through cerebellar degeneration [152]. βIII deletion in mice results in similar abnormalities, with altered organellar structure in Purkinje neurons and mislocalization of several synaptic proteins [153]. One SCA5 mutation appears to disrupt the βIII–Arp1 interaction, and results in an excess accumulation of βIII on the Golgi [154]. These features might lead to a model in which βIII spectrin coordinates efficient post-Golgi cargo sorting through interactions with dynactin.

ER–Golgi Transport

ER-synthesized 'cargo' proteins destined for secretion or delivery to the plasma membrane or other organelles get packaged into anterograde vesicles at specific ERES using the coatomer II (COPII) machinery [95,155]. These vesicles ultimately get delivered to *cis*-Golgi. ER-resident proteins that erroneously made this trip get returned to the ER by packaging into retrograde-transporting COPI vesicles [95,155].

In mammals, the distance between ERES and *cis*-Golgi is extremely heterogeneous and can be large. Consider a HeLa cell, which contains many ERES scattered all over the cell, but one central Golgi [156]. Proteins packaged in all ERES must get to the Golgi, which is between 1 μm to >20 μm away — how does this occur efficiently?

One possible answer is motor-based transport along microtubules, starting at the ERGIC (also called the VTC), which lies between ER and the Golgi [95,155]. The ERGIC is an irregular conglomeration of vesicles and tubules that can be flattened and highly branched, and debate exists as to whether this is a stable or transient structure. A central ERGIC is often present near the Golgi, with smaller peripheral ERGICs near peripheral ERES [157]. Dynein-based transport along microtubules plays a role in anterograde movement of vesicles between peripheral ERGICs and the central *cis*-Golgi [158], while several kinesins might be involved in Golgi-to-ERGIC retrograde transport [159].

The directionality of the motors implies that microtubule plus ends should be near ERGIC, while minus ends should be near *cis*-Golgi. This situation seems reasonable since the Golgi is near the MTOC in many mammalian cells. However, the microtubules nucleated from the *cis*-Golgi [16] might be direct routes for trafficking to or from the ERGIC (Figure 6).

Does actin play a role in ER-to-Golgi transport? An intriguing possibility is presented by the protein WHAMM (which stands for WASP homologue associated with actin, microtubules and membranes). WHAMM localizes to the ERGIC, *cis*-Golgi, and microtubule-associated ERGIC-derived structures that might be transport intermediates [113]. WHAMM suppression compromises anterograde ER-to-Golgi trafficking [113]. As its name suggests, WHAMM contains motifs that bind both microtubules and membranes directly. WHAMM's association with actin is through its

ability to activate Arp2/3 complex [113]. Thus, WHAMM might mediate an actin- and microtubule-dependent transport step between ERGIC and *cis*-Golgi. What this step might be is unclear, but WHAMM potently tubulates membranes, in a manner dependent on its activation of Arp2/3 complex [113]. Given that the ERGIC and ERGIC-derived membranes can be highly tubulated, WHAMM might contribute to their morphogenesis or transport.

ER–Golgi Transport in Neurons

Neuronal dendrites represent a fascinating variation of the typical secretory pathway. While the bulk of ERES and ERGIC and the main Golgi apparatus resides in the cell body, dendrites also contain significant ERES and ERGIC, in addition to small 'Golgi outposts', which enrich at dendritic branch points [84,85,160]. Protein secretion can take two routes from dendritic ERES: retrograde transport back to the central Golgi; or transport to dendritic Golgi outposts. The fact that most cargo goes back to the central Golgi [85] and that only a subset of dendrites contains Golgi outposts [160] suggests that secretion through Golgi outposts might represent a pathway for specific cargo or situations [160]. The role of the cytoskeleton in this specialized secretory pathway is unclear. Golgi outposts are capable of nucleating microtubules [151], but the majority of acentrosomal microtubules might originate elsewhere [161].

Conclusions and Future Perspectives

We envision that mechanistic connections between cytoskeleton and ER/Golgi will be clarified significantly in the near future, in terms of the molecules involved, their roles (in translocation or deformation of membranes) and their interactions with known components or regulators of organelle structure/function. Particularly intriguing are connections between the cytoskeleton and 'membrane-shaping' proteins, such as reticulons/REEPs and Golgi matrix proteins, since these literally coat sections of their respective organelles. The development of super-resolution microscopy and a greater appreciation for EM preservation of both organelles and cytoskeleton will reveal ultrastructural relationships in a fundamentally new manner. We hope that this review helps to clarify our current understanding of the relationship between the cytoskeleton and ER/Golgi, by inserting new pieces into the puzzle. While some of our pieces might be inserted somewhat incorrectly, we hope that their presence will allow others to replace them with better-fitting ones.

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