The role of microtubules in transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells

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Abstract

The organization of intracellular compartments and the transfer of components between them are central to the correct functioning of mammalian cells. Proteins and lipids are transferred between compartments by the formation, movement and subsequent specific fusion of transport intermediates. These vesicles and membrane clusters must be coupled to the cytoskeleton and to motor proteins that drive motility. Anterograde ER (endoplasmic reticulum)-to-Golgi transport, and the converse step of retrograde traffic from the Golgi to the ER, are now known to involve coupling of membranes to the microtubule cytoskeleton. Here we shall discuss our current understanding of the mechanisms that link membrane traffic in the early secretory pathway to the microtubule cytoskeleton in mammalian cells. Recent data have also provided molecular detail of functional co-ordination of motor proteins to specify directionality, as well as mechanisms for regulating motor activity by protein phosphorylation.

Introduction

The intracellular organization of most cells is highly dependent upon the presence of a functional cytoskeleton. Actin, microtubule and intermediate filaments provide frameworks for generating tension, are plastic to allow highly organized changes in cell or organelle shape [1], and can be used for the positioning of intracellular organelles and the movement of intracellular components between

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From ground-breaking experiments using yeast genetics and biochemistry, we now know an extraordinary amount about the machinery involved in the transport of protein and lipid between the ER and Golgi [6,7]. Partly as a consequence of the very different organization of yeast and mammalian cells (for example, see [8]), little is known about the mechanisms used by animal cells to coordinate membrane traffic using the microtubule cytoskeleton. Key differences have been found in the way in which plants, animals and lower eukaryotes utilize cytoskeletal components. In the context of the secretory pathway, plants are much more dependent upon the actin cytoskeleton than on microtubules [9,10]. In mammalian cells, both have a significant role to play [5,11]. However, an intact microtubule network is not required for transport through the secretory pathway, but it does greatly enhance its efficiency [12,13]. Furthermore, it is likely to be of much more significance in highly organized cell types, such as neurons [14]. This review will focus on the role of the microtubule network in the control of intracellular membrane traffic in mammalian cells.

The organization of the early secretory pathway

Intracellular organization is determined in part by the polarity of the microtubule cytoskeleton. Rapidly growing plus-ends are generally located in the cell periphery with the more slowly growing minus-ends at a central, juxtanuclear location (Figure 1). One area that has been studied extensively is the positioning of the ER and Golgi apparatus in cells [11,15–18]. The ER is a large membrane-bound organelle that consists of an extensive network of interconnecting tubules and cisternae that spread peripherally along microtubules throughout the cytoplasm [19]. This membrane system is subdivided into regions that are covered with ribosomes (referred to as rough ER) and regions devoid of ribosomes (referred to as smooth ER). The rough ER is the site of translocation of secretory cargo across the membrane, the integration of transmembrane proteins into the membrane, the synthesis, modification and degradation of proteins, and lipid biogenesis. The smooth ER is primarily involved in calcium storage/release. Specialized regions of the smooth ER known as transitional elements, tER (transitional ER) or ER exit sites also exist, and are the sites of secretory cargo exit from the ER [20,21]. Proteins are concentrated into ER exit sites by the action of the COPII (coatamer protein II) coat complex (reviewed in [22]). Subsequent budding of cargo-containing vesicles is followed rapidly by their uncoating and accumulation into a VTC (vesicular tubular cluster) that acts as the transport intermediate for traffic to the Golgi [20,23–25]. This scheme is illustrated in Figure 1. The network of ER membranes is distributed throughout the cell [26]. ER membranes interact with microtubules in a number of ways and are pulled towards the cell periphery by the plus-ended motor, kinesin [27]. Microtubule tips have also been shown to push and pull the ER into networks [15,16,28]. One functional consequence of this is that there can be large distances (tens of microns) across which transport intermediates might have to travel, and it is in this respect that the microtubule cytoskeleton becomes central to the efficiency of membrane traffic between the ER and Golgi.
The Golgi occupies a central location in most mammalian cells, lying at the microtubule-organizing centre in mammalian cells and both its positioning and orientation (as it is a polarized structure) are controlled by the cytoskeleton [11,18,29]. The Golgi apparatus is a highly dynamic organelle that occupies a central position in the eukaryotic secretory pathway [18,30]. Nocodazole causes the disruption of microtubules and consequently the fragmentation of the Golgi apparatus [29]. These Golgi elements remain polarized and, following washout of nocodazole, translocate along microtubules towards minus-ends located at the centre of the cell [31]. The role of microtubules in Golgi organization and function is dealt with extensively in an excellent recent review [11] and is not considered further here.

Machinery molecules involved in the transport of cargo from the ER to the Golgi [such as SNAREs (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor)] are recycled back to the ER [6] in a COPI (coatamer protein I)-dependent manner [32]. Other molecules utilize a second, COPI-independent, pathway [33,34]. This retrieval mechanism is also used to direct ‘ER-resident’ proteins back to the ER should they have exited with...
cargo. An ever-increasing amount of data suggests that both the anterograde (Golgi-directed) and this retrograde (retrieval) pathway use the microtubule cytoskeleton [25,35].

**Microtubule-based motors: kinesin, dynein and dynactin**

Molecular motor proteins have the specialized task of converting chemical energy from ATP hydrolysis into force and motion, which is used to drive a wide variety of cellular processes, including the unidirectional transport of molecules and organelles along cytoskeletal tracks. Three superfamilies of motor proteins have so far been identified: kinesins, the majority of which are plus-end-directed microtubule motors, dyneins, which are minus-end-directed microtubule motors, and myosins, which are actin-dependent motors [36,37]. Recent genomic and functional studies suggest that motor proteins in several organisms (fungi, parasites, plants and animals) stem from one of five cargo-carrying motors, the proposed ‘toolbox motors’ [36]. These proteins, which include three microtubule plus-end-directed kinesins (conventional kinesin, kinesin II and Unc104/KIF1A), cytoplasmic dynein and myosin V, have evolved through gene duplication, alternative splicing and the addition of associated subunits to provide different force-generating functions required with increasing cellular complexity [36]. This review will specifically detail those involved in the transport of membranes within the early secretory pathway of mammalian cells, and the reader is referred to other reviews for further details of motor protein mechanics and function (e.g. [14,38–40]).

Conventional kinesin [41], also referred to as kinesin I or KIF5, is found in many species of animals, and filamentous and fission fungi, but not in budding yeast [42]. This motor protein exists as a homodimer of heavy chains with two flanking light chains that appear to be absent in filamentous fungi [43]. The heavy chains are organized into distinct domains: the N-terminal motor domain, which contains the nucleotide- and microtubule-binding sites, the extended coiled-coil domain interrupted by a central hinge region, and the C-terminal globular tail domain thought to promote light chain and/or cargo binding (see [40] for a review of kinesin structure). The light chains have also been implicated in cargo binding [40], although one study suggests that they are unnecessary for this function [44]. Kinesin II (KIF3) is assembled from two distinct motor subunits that heterodimerize through charge interactions in an extended region of the coiled-coil stalk domain [40]. There is also an additional accessory subunit known as KAP (kinesin-associated protein) that is bound to the motor’s C-terminal tail, and for this reason, the motor is also referred to as heterotrimeric kinesin II [40]. Kinesin is centrally involved in ER membrane movement [15] and in the transport of proteins from the Golgi to the ER [45], and has also been localized to ER exit sites [46] (Figure 1). Kinesin II has been localized by immunofluorescence and immunoelectron microscopy to KDEL (Lys-Asp-Glu-Leu)-receptor-containing transport intermediates in *Xenopus* cells [47].

Cytoplasmic dynein is a large multiprotein complex of 1.2 MDa, which drives motility towards the minus-ends of microtubules [48]. The complex
comprises two identical heavy chains (DHCs), four light intermediate chains (DLICs), two intermediate chains (DICs) and a number of light chains (DLCs) with molecular masses of between 10 and 13 kDa (see [49] for a review). In humans, there are two DHCs (CD1 and CD2) which intriguingly can both be localized to the Golgi apparatus [50,51]. Multiple isoforms of these subunits exist, giving rise to multiple species of dynein, differing in their subunit composition [52]. This isoform diversity supports a key role for these subunits in dynein function, such as regulation of activity or cargo binding. The combinatorial presence or absence of these accessory subunits is likely to be central to the definition of cytoplasmic dynein function in cells.

Dynein-based motility is enhanced by a 1.2 MDa multiprotein protein complex called dynactin (‘dynein activator’) [53]. Dynactin comprises ten distinct polypeptide subunits, and contributes to cytoplasmic dynein function by acting as an ‘adapter’, expanding the number and types of cargo dynein can transport and by enhancing motor processivity [54]. Attachment to dynein occurs through direct association between the N-terminus of DIC to the central region of the dynactin subunit p150glued [55]. The p150<sub>glued</sub>, p50<sub>dynamitin</sub> and p24 subunits form a structurally distinct side-arm that binds strongly to microtubules via p150<sub>glued</sub> [56]. The remaining dynactin subunits form a filament-like structure that comprises the actin-related protein Arp1, conventional actin, α- and β-actin-capping proteins, and p62, p27 and p25 [57]. This filament, referred to as the Arp1 filament is thought to mediate attachment to membranes through interactions with spectrin [58]. Disrupting the dynein–dynactin interaction with specific inhibitory antibodies disrupts dynein-based motility [59]. Overexpression of individual dynactin components causes specific changes in cellular function that are well documented [60]. For example, overexpression of p50<sub>dynamitin</sub> disrupts microtubule organization [60] and mitosis [61], and causes Golgi fragmentation, as well as disrupting the localization of endosomes and lysosomes in interphase cells [59–61]. Overexpression of p150<sub>glued</sub> [60] results in dissociation of the p150<sub>glued</sub>-containing side-arm from the remainder of the dynactin complex, uncoupling microtubule binding and cargo-attachment functions.

**Recruitment to membranes**

Clearly, the central point of co-ordination of membrane traffic by the cytoskeleton is the means of attachment of motors and their regulators, such as dynactin, to membranes [62]. Although motor proteins might associate with several membranous organelles and mediate a wide range of intracellular transport steps, the nature and identity of the motor–cargo interactions remains particularly elusive. In particular, the specificity of motor binding in both space and time is a highly complex process that must be tightly regulated. It is likely that, in many cases, both the recruitment and activity of motors will be co-ordinated. Associated accessory subunits can often mediate cargo interactions and cargo-bound ‘receptor’ proteins such as dynactin [62] or kinectin [63] could also mediate motor-protein attachment. This area is also of increasing interest in terms of target discovery in the pharmaceutical industry [64].
Rab proteins act as major co-ordinators of intracellular membrane traffic [65]. Considerable evidence now exists that Rabs are primary recruiters of motors to membranes [66]. Rab6 localizes to the cytoplasmic face of the Golgi, and is involved in both endosome-to-Golgi [67] and Golgi-to-ER transport [33,34,68]. Rab6 has been shown to regulate recruitment of dynein to membranes (Figure 1), while other Golgi-localized Rabs do not [69]. The interaction between Rab6 and the dynein-dynactin complexes is regulated by BicD (Bicaudal D). BicD co-localizes and can be co-immunoprecipitated with dynein and dynactin, and has been shown to interact with the dynactin subunit p50dynamitin [70]. Furthermore, overexpression of BicD increases the recruitment of dynein and dynactin to Rab6-containing vesicles [71]. Overexpression of a C-terminal fragment of BicD that can bind to Rab6 (but not to the motor complex) inhibits the movement of Rab6-labelled vesicles [71]. BicD has been shown to bind only to the active form of Rab6, providing the basis for a molecular switch for the transport of activated Rab6-decorated vesicles [71].

Other polypeptides that associate with dynactin have also been shown to play a role in membrane traffic. Lis1 (lissencephaly 1) was initially identified as a non-catalytic subunit of brain platelet-activating factor acetylhydrolase [72], but has since been shown to interact with cytoplasmic dynein and dynactin [73,74]. Nudel (NudE-like), a mammalian homologue of the *Aspergillus* dynein regulator NudE, is functionally involved in the transport of proteins that contribute to the inactivation of the spindle checkpoint in mitosis [75,76]. Lis1 and Nudel appear to regulate cytoplasmic dynein in neuronal migration and mitosis through a direct interaction [77]. Expression of Nudel mutants unable to bind to either Lis1 or DHC1, or depletion of Nudel using RNA interference, cause fragmentation/dispersion of the Golgi cisternae, lysosomes and endosomes [77]. Dispersion of the typical juxtanuclear localization of the ER–Golgi intermediate compartment was also observed [77]. Other modulators of dynein function that may have a role in transport within the early secretory pathway have also been identified. ZW10 interacts with p50dynamitin, and has recently been shown to play a role in membrane trafficking between the ER and the Golgi [78]. Specifically, ZW10 binds to the ER-localized t-SNARE (target SNARE), syntaxin 18, and its overexpression results in disorganization of the early secretory pathway and disruption of anterograde and retrograde transport [78].

**Evidence for the role of motors in membrane traffic between the ER and Golgi**

In addition to the role of the cytoskeleton in the positioning of the ER and Golgi, the movement of membrane-bound structures (vesicles and tubules) between the ER and Golgi has received much interest of late. There is now considerable evidence for a direct role for the microtubule cytoskeleton in the co-ordination of traffic between the ER and Golgi [11,79], and in the generation and maintenance of the steady-state localization of components of the pathway [18,29]. Live-cell imaging of the COPII complex has also shown that depolymerization of microtubules with nocodazole results in an enlargement of the size of COPII-coated ER exit sites and an inhibition of their mobility [80]. COPII-
coated ER exit sites are positioned along microtubules in skeletal muscle fibres [81], and are reorganized upon muscle cell differentiation [82]. This microtubule-dependent reorganization results in the localization of ER exit sites to the minus-ends of microtubules in myotubes. Thus there is a clear interdependence of the sites of VTC formation and the microtubule cytoskeleton. A clear correlation also exists between ER exit sites and the microtubule cytoskeleton in fibroblast and epithelial cells (D.J. Stephens, unpublished observations; and Figure 2). COPII-coated ER exit sites accumulate in the juxtanuclear area (in direct proximity to Golgi membranes) following expression of Sar1p–GTP [83,84], and also intriguingly as cells proceed through the cell cycle [85].

Visualization of ER-to-Golgi transport by electron microscopy [23] and time-lapse microscopy of live cells [25] strongly implicates a role for microtubules in ER-to-Golgi transport. Live-cell imaging of GFP (green fluorescent protein)-tagged cargo molecules has shown that translocation of VTCs occurs along curvilinear tracks at speeds consistent with microtubule-based motility [24,25]. Inhibition of dynein-mediated transport by overexpression of the p50dynamitin subunit of dynactin inhibits the transport of these structures [25] by blocking recruitment of dynein to these membranes [50]. Somewhat surprisingly, kinesin is recruited to ER exit sites upon incubation with a GTP-restricted mutant of Sar1p [46], a small GTPase that initiates COPII complex assembly at ER exit sites [22]. Given the minus-end-directed destina-
tion of ER-to-Golgi transport carriers, this recruitment of kinesin may reflect a role in positioning of ER exit sites rather than a direct role in secretory cargo export. The role of motors in these processes is illustrated by Figure 1. The role of kinesins with respect to COPII [46] may be in the initial deformation of the ER membrane or organization of ER export by direct association of export sites with microtubules. Alternatively, or in addition, the recruitment of kinesin at this point may be related to its later function in retrograde (Golgi-to-ER) transport [45]. This would be consistent with a model in which both plus- and minus-end-directed motors are present on VTCs, with directionality of transport being controlled by regulation of these two motor populations.

Our working hypothesis is that a direct interaction between COPII-coated ER exit sites and microtubules co-ordinates the initial stages of ER-to-Golgi transport, establishing this functional link at an early stage prior to microtubule-based transport of membranes to the Golgi [86]. There are two principal modes that could be envisaged for a mechanism of ER exit site capture by microtubules. First, that the ends of microtubules associate with ER exit sites either during growth or shrinkage. Alternatively, that ER exit sites associate laterally with microtubules independent of changes in length. This second mechanism might act independently of the presence of dynactin at plus-ends, and may depend on recruitment of dynactin to membranes prior to its binding to microtubules. Intriguingly, a pool of p150glued has recently been shown to be involved in capture of membranes during reformation of the Golgi apparatus following removal of brefeldin A from treated cells [86]. Brefeldin A causes a redistribution of proteins that are normally localized within Golgi cisternae to the ER [87]. This effect is reversible, allowing reformation of the Golgi by an ER-to-Golgi transport process. Vaughan et al. [86] observed that membrane clusters containing Golgi enzymes transiently associated with p150glued at the plus-ends of microtubules during Golgi reformation after brefeldin A washout. This process was also shown to be regulated by phosphorylation of p150glued, suggesting a mechanism for controlling capture and/or motility of VTCs. One can envisage that this could easily be linked to some quality-control mechanism operating to ensure productive VTC formation.

Directionality and regulation

Directionality is a vital concept in intracellular transport. Membranes (vesicles and/or tubules) must be linked to the microtubule, motor proteins must be recruited and their activity regulated. The regulation of motors is particularly important in the context of directionality of membrane traffic. A considerable amount of recent data has revealed the presence of multiple motors on intracellular structures, with the regulation of the balance between plus- and minus-end-directed motors resulting in directed transport [88–90]. Time-lapse-imaging experiments have revealed that many cargo proteins move bidirectionally along cytoskeletal tracks and frequently change their direction (e.g. see [24,25]). Yet, since motor proteins only move in a unidirectional manner either towards the plus- or minus-ends of microtubules, how is bidirectional transport achieved? What are the controlling factors that determine direction? Two models have been
proposed: the ‘tug-of-war’ scenario and the ‘motor co-ordination’ model [89,91]. The first model, as the name suggests, involves a struggle between opposing motors bound to the same cargo molecule. The winning motor type determines the direction of transport, and regulation could involve altering force production, enzymic activity or changing the type and number of active motor proteins bound to the cargo. The second model relies on motor protein co-ordination [89], and in this instance, opposite-polarity motors are co-ordinated in such a way that they do not interfere with each other’s function. Regulating the ‘state’ (on or off) that the motor proteins are in determines directionality simply by keeping one set of motors ‘on’ for longer. Data exist in favour of both of these models, and it is possible that both operate under different scenarios in different cell types. The co-ordination model is supported by work on *Drosophila* embryos in which the transport of lipid droplets was monitored following perturbation of minus-end-directed movement [89]. Somewhat surprisingly, plus-end-directed movement was also found to be inhibited. In the tug-of-war model, one would expect this mode to predominate, but instead this provides evidence in support of co-ordination of minus- and plus-end transport. Indeed, directionality determinants have been identified in this system [89,92,93]: dynactin has a role to play [89], as do other newly identified proteins such as klarsicht and halo [92,93]. Together these factors prevent the onset of a tug-of-war scenario through regulation of individual motor activity. Although much of this work relates to *Drosophila* motor function, much of it is directly relevant to mammalian systems.

In further support of motor co-ordination, GFP–dynein has been shown to remain bound to cargo even when that cargo switches direction [90]. Further work using *Xenopus* melanophores has provided the first physical evidence that dynactin, known to link cytoplasmic dynein to membranes, also connects kinesin II to cargo molecules [94]. This dual interaction was shown to occur through the direct binding of the p150\(^{\text{glued}}\) dynactin subunit to either DIC or the accessory protein KAP [94]. Binding was also found to be exclusive and competitive, with both DIC and KAP associating to similar regions on the p150\(^{\text{glued}}\) protein (residues 600–811). This suggests that the DIC/KAP-binding site on p150\(^{\text{glued}}\) is probably central to the control of directionality. Since binding is competitive, these data could be seen to support the tug-of-war model, but in fact can equally be integrated into a motor co-ordination model. One simply needs to invoke the presence of additional directionality determinant acting in concert with p150\(^{\text{glued}}\). It is likely that many of the mechanisms coordinating the activity of opposite polarity motors are applicable in other systems controlling organelle positioning as well as motility of transport intermediates in the early secretory pathway.

**Regulation by phosphorylation**

Mechanisms that are likely to regulate the activity of microtubule-based motor proteins include conformational changes, protein phosphorylation and isoform variability. Both the heavy and light chains of kinesin are phosphorylated *in vivo* on serine residues [95,96]; these phosphorylation events appear to regulate membrane association [97] and motor activity [98], but their precise
significance remains unclear. Dynein and dynactin are also subject to regulation by phosphorylation [99–103]. Similarly, experiments using dynactin-null mutants of Neurospora have revealed dynactin-dependent phosphorylation of the DLCs, thereby regulating dynein ATPase activity [103]. Other in vitro assays have suggested that increased phosphorylation of DICs and DHCs results in an inhibition of ATPase activity [104]. Both DICs and p150\textsuperscript{glued} have been found to be subject to cell-cycle regulation, becoming hyperphosphorylated during metaphase in HeLa cells [99]. This is likely to control the association of these complexes with membranes [102]. DIC phosphorylation has been implicated in the regulation of the all-important dynein–dynactin interaction [105]. Phosphorylation of DIC Ser\textsuperscript{84} has been shown (by site-directed mutagenesis) to diminish p150\textsuperscript{glued} binding and thereby reduce the affinity of dynein for dynactin [105]. More recently, binding of the dynactin subunit p150\textsuperscript{glued} to elongating microtubule plus-ends has been shown to be subject to regulation by phosphorylation [86]. A phospho-sensitive monoclonal antibody identified Ser\textsuperscript{19} as the site of p150\textsuperscript{glued} phosphorylation. Stimulation of PKA (protein kinase A) with forskolin treatment promoted p150\textsuperscript{glued} phosphorylation in COS-7 cells and reduce microtubule binding [86]. Phosphorylation site mutants further reinforced this with a Ser\textsuperscript{19}→Glu point mutant, mimicking phosphorylated p150\textsuperscript{glued}, showing diminished microtubule association, while the Ser\textsuperscript{19}→Ala mutant promoted microtubule binding. Live-cell imaging also revealed that a transient association of p150\textsuperscript{glued}-labelled microtubules with membranes occurred prior to minus-end-directed transport [86]. This led to a model in which phosphorylation of p150\textsuperscript{glued} controls its association with microtubules: the non-phosphorylated form preventing motility by allowing microtubule binding (acting as a brake) with phosphorylation triggering release from the microtubule and subsequent dynein-mediated minus-end motility [86]. Furthermore, these data implicate dynactin (and specifically p150\textsuperscript{glued}) in a ‘search and capture’ mechanism [86], directing the initial stages of coupling of membranes to microtubules prior to minus-end-directed movement of Golgi-directed membranes. These data strongly suggest that dynactin is directly coupled to the membranes of the early secretory pathway and that its phosphorylation controls the efficient transport of intermediates between the ER and Golgi. The mechanisms defining recruitment of these motor complexes and accessory proteins to membranes of the early secretory pathway remain unknown.

Conclusions and perspectives

Our understanding of the molecular mechanisms that integrate membrane traffic with the cytoskeleton is increasing steadily. More is now known about the mechanisms coupling individual membranes to microtubules, but many questions still remain unanswered. Are there independent cargo adaptors for each membrane/vesicle type? How and when are these adaptors (such as dynactin) and motors recruited? How initiation of motility is coupled with the formation of productive transport carriers remains unclear. Do quality-control mechanisms ensure that all relevant cargo and machinery are included prior to
the initiation of transport? Our knowledge of how motors are regulated (for example, by phosphorylation or subunit composition) now needs to be coupled with our knowledge of the organization and directionality of individual membrane traffic events.

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References


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