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Microtubule motors at the intersection of trafficking and transport

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Molecular motors drive the transport of vesicles and organelles within the cell. Traditionally, these transport processes have been considered separately from membrane trafficking events, such as regulated budding and fusion. However, recent progress has revealed mechanistic links that integrate these processes within the cell. Rab proteins, which function as key regulators of intracellular trafficking, have now been shown to recruit specific motors to organelle membranes. Rab-independent recruitment of motors by adaptor or scaffolding proteins is also a key mechanism. Once recruited to vesicles and organelles, these motors can then drive directed transport; this directed transport could in turn affect the efficiency of trafficking events. Here, we discuss this coordinated regulation of trafficking and transport, which provides a powerful mechanism for temporal and spatial control of cellular dynamics.

Introduction

The story of the blind men and the elephant is an apt metaphor for cell biologists studying either vesicular trafficking or intracellular transport. In this story, each wise man describes an elephant from only the parts that he himself experiences, such as trunk or tail, without seeing how these aspects relate to a larger whole. Although many of the steps in intracellular trafficking depend on active transport along the cytoskeleton, studies on the coordinate regulation of trafficking from one compartment to another have often proceeded separately from the analysis of the mechanics of motor-driven transport. Two key developments have enabled us to start to see how some parts of the elephant are related: live cell studies, which provide temporal and spatial resolution of trafficking and transport events, and reconstitution of vesicle motility *in vitro*, which enables the exploration of regulatory mechanisms. Together, these approaches have led to the conclusion that regulatory and scaffolding or adaptor proteins that govern trafficking events could also mediate recruitment of motor proteins to vesicles and organelles, thus providing coordinated control of these processes. Here, we highlight the clarity these studies have brought to the mechanisms that regulate membrane dynamics along the microtubule cytoskeleton. Transport along actin filaments is also crucial in intracellular trafficking but is not discussed here; see a recent review by Krendel and Mooseker [1].

Kinesin and dynein motors drive intracellular transport

Microtubules form a dynamic and polarized cytoskeleton. In most cell types, microtubule plus ends grow dynamically out from the microtubule-organizing center (MTOC), where the minus ends are more stably tethered (Figure 1). Two major superfamilies of microtubule motor proteins have been identified, kinesins and dyneins (Box 1). Kinesins are an extended superfamily, with up to 45 members expressed in mammalian cells; different kinesins share a common motor domain but diverge considerably in their cargo-binding tail domains [2]. By contrast, one major form of cytoplasmic dynein transports many different cargos in the cell [3]. Most dynein-mediated functions require an additional accessory complex known as dynactin, which functions in both cargo binding and motor processivity [4].

Which way to go: directionality of transport processes

Conventional kinesin (now known as kinesin-1 [2]) and most of the other vesicle motors in the kinesin superfamily move unidirectionally toward the plus end of the microtubule [5]. Thus, kinesins are likely to be involved in trafficking events directed towards the cell periphery, such as motility from the Golgi to the plasma membrane. However, minus end-directed kinesins also contribute to intracellular trafficking events, such as the minus end-directed transport of early endosomes [6]. In general, kinesins are processive motors, taking long runs along the microtubule before detaching. This leads to highly efficient transport of cargo. Kinesins are also relatively powerful, generating up to 6 pN of force per motor [5], so relatively few active motors are necessary to move cargo effectively [7]. This could enable more precise regulation of motor activity in the cell or more rapid changes in direction of transport.

Cytoplasmic dynein is the major motor for the minus end-directed transport of vesicles and organelles along microtubules, driving movement toward the cell center, such as traffic from the endoplasmic reticulum (ER) to the Golgi. Dynein is also a processive motor; in association

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Box 1. The microtubule motors that drive intracellular trafficking

Kinesins

Kinesins are an extended superfamily, with up to 45 members expressed in mammalian cells [2]. These proteins share a homologous domain that binds to the microtubule in an ATP-sensitive manner. In many of the kinesins, this domain is necessary and sufficient for motor function. In other members of the kinesin superfamily, such as the kinesin-13 family member MCAK, the conserved 'motor' domain functions as a microtubule depolymerase [5].

For active motors in the kinesin superfamily, the motor domain is fused to a neck linker domain that actively participates in force production, a coiled-coil domain that could mediate association with other subunits, and a cargo-binding domain. Although the motor domains share a relatively high degree of homology, there is considerable variation in the accessory subunits and cargo binding domains [5]. This extensive variability means that kinesins can be functionally specific in the cell, specialized for the transport of individual cargos. There is also variability in motor direction: whereas most kinesin family motors, such as kinesin-1, move toward the plus end of the microtubule, several members of the superfamily, such as kinesin-14 family member KIFC2, drive transport toward the minus end of the microtubule.

Cytoplasmic dynein and dynactin

Cytoplasmic dynein is a large and complex motor protein composed of two heavy chains, two intermediate chains and additional light intermediate and light chains [3]. The dynein heavy chain is a member of the AAA family of ATPases and folds to form a heptameric ring with a short coiled-coil extension that binds to the microtubule and an extended tail domain that dimerizes with a second heavy chain and also binds to the intermediate and light subunits. There are multiple consensus ATP binding sites within the AAA domain of the dynein head, but hydrolysis of ATP at a single site (AAA1) is necessary and sufficient to drive motility along the microtubule [53].

There is a single gene encoding the heavy chain of cytoplasmic dynein 1, resulting in one major form of the enzyme expressed in eukaryotic cells. However, multiple genes encode intermediate, light intermediate and light chains, which could provide some functional specificity for cargo interactions [3]. A distinct gene encodes the heavy chain of cytoplasmic dynein 2. This polypeptide homodimerizes to form a distinct complex involved in intraflagellar transport [3].

Dynactin is a large accessory complex for cytoplasmic dynein, composed of eleven subunits [4], including the $p150^{Glued}$ subunit that binds to dynein, to the microtubule and to the Arp1 subunit that assembles to form an actin-like filament at the base of the complex [4]. Dynactin enhances the processivity of the dynein motor and also mediates some interactions with cargo [4].

Cytoplasmic dynein is the major motor for minus end-directed transport of organelles along cellular microtubules, and in motility assays *in vitro* with multiple dynein motors, dynein moves exclusively to the microtubule minus end. However, recent studies on single dynein–dynactin motor complexes under conditions of no load reveal that although the overall bias in direction is toward the microtubule minus end, the dynein–dynactin complex can move bidirectionally [54], including processive motility towards either end of the microtubule [55]. This surprising observation might reflect the intrinsic flexibility of the dynein structure, as both the linkage between the motor domain and tail domain and the tail domain itself seem surprisingly flexible [56]. This flexibility and the ability of dynein to move from protofilament to protofilament across the surface of the microtubule and bidirectionally along the length of the microtubule could enable dynein to bypass obstacles during transport within the cell [55].

with dynactin, which has an independent microtubule-binding site, the lengths of the dynein runs can be increased further [4]. Dynein generates up to 1.1 pN of force per motor [8], significantly less than kinesin, so that more dyneins than kinesins are required to move a similar cargo. On average, it has been estimated that there are between 2 and 5 active motors associated with vesicular cargo moving toward either the plus or minus end of the microtubule [7].

Although some vesicles and organelles may be transported in a smoothly unidirectional manner through the cell, many others move bidirectionally [7]. Excursions in one direction can be interrupted by pauses or movements in the opposite direction. This bidirectional motility is thought to result from the concurrent binding of oppositely oriented motors, either multiple kinesins or both dynein and kinesin, that are coordinately regulated [7,9,10]. The regulatory mechanisms that govern this coordination are not yet clear but might involve the direct coupling of motors [11], or coordination through accessory factors such as dynactin [12].

What goes where: motor–cargo interactions

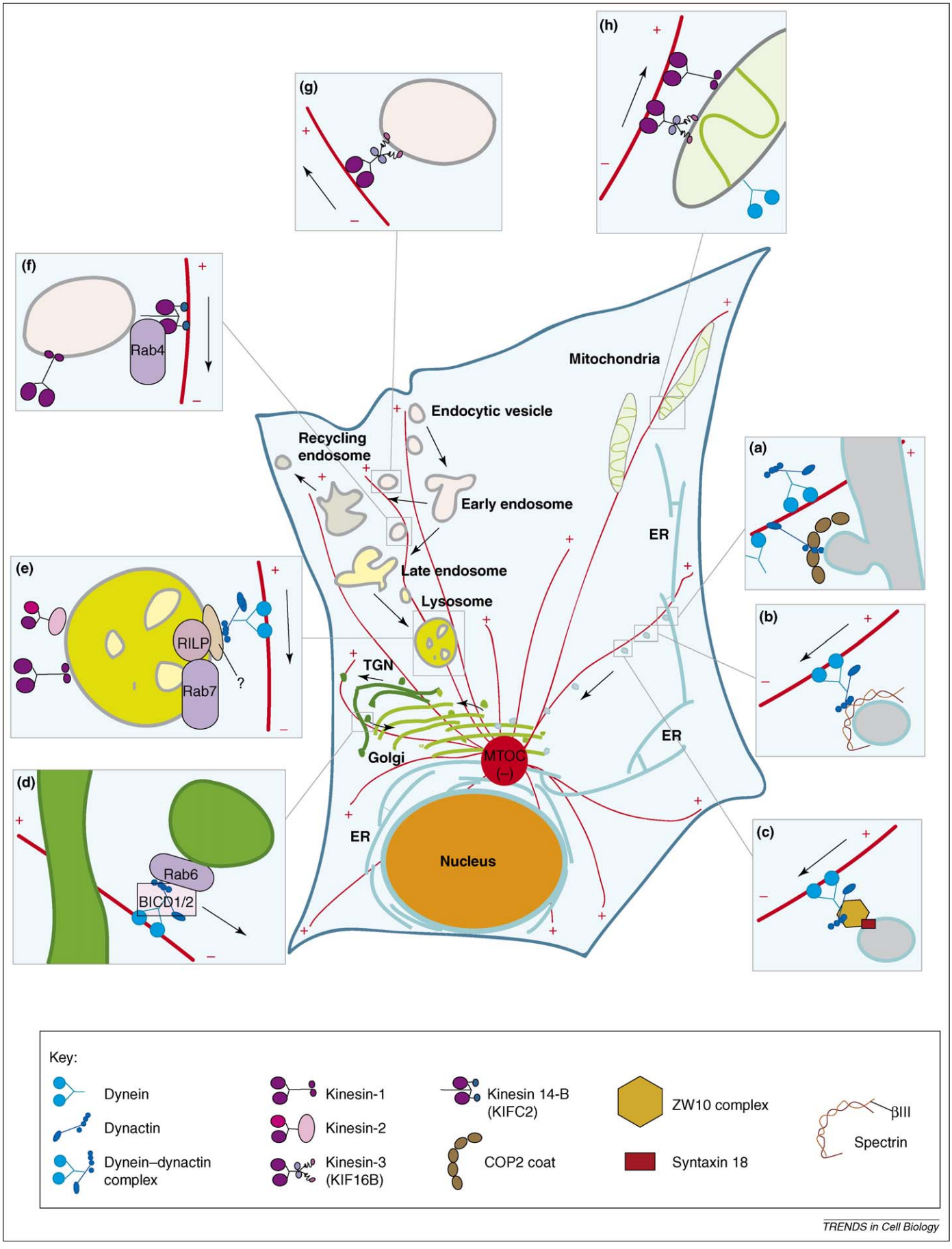
Deciphering motor–cargo interactions for intracellular vesicles and organelles is an on-going process. Initial models suggesting that there might be a direct and simple association between a motor and a membrane-bound receptor have not held up. Instead, many motors seem to interact with cargo through indirect associations, through one or more adaptor or scaffolding proteins. A striking example of this type of association is the interaction of the kinesin-2 family motor KIF17 with its vesicle-bound cargo, the NR2B subunit of the *N*-methyl-D-aspartic acid (NMDA) receptor. KIF17 interacts indirectly with this cargo in an association mediated by three adaptor proteins, mLin-10, mLin-2 and mLin-7 [13]. Similarly, the scaffold protein gephyrin links the glycine receptor to cytoplasmic dynein [14].

Alternatively, motors might associate with membrane lipids, either directly or indirectly. For example, the kinesin-3 family member UNC-104 binds to phosphatidylinositol(4,5)bisphosphate (PtdIns(4,5)P_2) through a pleckstrin homology (PH) domain in the C-terminal tail of the motor [15]. Dynein has also been proposed to associate with membrane lipids but more indirectly, through dynactin and the cytoskeletal protein spectrin. The actin-like Arp1 filament of dynactin binds to an organelle-associated isoform of spectrin, which is recruited to membranes such as those of the Golgi through its PH domain [16,17].

Co-regulation of trafficking and transport

One of the most interesting developments in the analysis of motor–cargo interactions and their roles in vesicular trafficking has been the identification of Rab proteins as potent regulatory molecules of motor protein recruitment. Rabs are monomeric GTPases that facilitate the specificity of vesicular trafficking [18]. Members of the Rab family are specifically targeted to intracellular vesicles and organelles, where they serve as molecular switches as they cycle between GTP-bound and GDP-bound forms, which each recruit a different set of effectors [19]. These binding partners participate in the regulation of vesicle formation and budding from donor compartments and in vesicle tethering or fusion with acceptor compartments.

Rabs also recruit molecular motors and therefore regulate transport between donor and acceptor compartments.


(h)
(g)
(f)
Rab4
Mitochondria
Endocytic vesicle
Recycling endosome
Early endosome
Late endosome
Lysosome
ER
(a)
(e)
RILP
Rab7
?
TGN
(b)
Golgi
MTOC (–)
ER
(d)
Rab6
BICD1/2
ER
Nucleus
(c)
Key:
Dynein
Dynactin
Dynein–dynactin complex
Kinesin-1
Kinesin-2
Kinesin-3 (KIF16B)
Kinesin 14-B (KIFC2)
COP2 coat
ZW10 complex
Syntaxin 18
βIII Spectrin
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A role for Rabs in regulating transport along the cytoskeleton was first identified in studies on the actin-based motor myosin-V. This motor is specifically recruited to melanosomes by the adaptor protein melanophilin and activated Rab27a [20]. Rabs have now been shown to participate in the recruitment of microtubule motors to membranes, including the recruitment of a kinesin to endosomes [21] and the recruitment of dynein to the trans-Golgi network (TGN) [22,23]. Rab-dependent recruitment of motors is a mechanism that enables precise spatial regulation of transport, as each Rab is localized to a specific cellular compartment. Rabs also provide temporal regulation, as binding to effector molecules is governed by the cycling between the GTP-bound and GDP-bound conformations [24]. As detailed later, this mechanism directly relates intracellular transport to the well-explored trafficking pathways of the cell.

There are also Rab-independent mechanisms of intracellular transport, in which motors are specifically recruited to cargo by scaffolding or adaptor proteins. Some of these interactions are regulated by other small GTPases. For example, the Rho-like GTPase Miro regulates the recruitment of kinesin to mitochondria by the protein Milton [25,26] (Box 2).

Alternatively, these interactions might be regulated by other signal transduction pathways in the cell, such as mitogen activated protein (MAP) kinase cascades, via scaffolding proteins. For example, Jun N-terminal kinase (JNK)-family interacting proteins have been implicated in the regulation of kinesin-mediated transport. JNK signaling regulates multiple processes in the cell, including the stress response pathway. JNK-binding scaffolding proteins (JIP1, JIP2 and JIP3) interact with kinesin-1 and might regulate kinesin-dependent transport [27–29]. Thus, there are multiple mechanisms that can contribute to the spatial and temporal regulation of motor localization and dynamics within the cell.

Participation of motors in intracellular trafficking pathways

ER-to-Golgi transport

The transport of vesicles from ER to Golgi along microtubules is dependent on the dynein–dynactin complex. The recruitment of the motor complex to vesicles exiting the ER is not fully understood, but could be facilitated by an initial interaction between dynactin and a component of the COPII coat that concentrates on cargo at ER exit sites (Figure 1a). Watson *et al.* [30] have proposed that dynactin is initially recruited to the COPII coat and that this recruitment enhances the export of cargo from the ER by mediating an interaction between the membrane and the microtubule. This membrane–microtubule association might further facilitate formation of the carrier compartment [30]. However, the association between dynactin and COPII is likely to be transient, as the COPII coat is shed before active transport to the Golgi [31] yet dynactin remains associated with the transport compartment. During shedding of the COPII, dynactin could be 'transferred' to an alternate membrane-associated protein, such as βIII spectrin [16,17] (Figure 1b), as the dynein–dynactin complex actively drives the motility of the transport vesicles to the perinuclear Golgi.

Another mechanism that might link dynein and dynactin to the ER-to-Golgi transport compartment involves a complex first identified through its role in the mitotic checkpoint machinery. This complex, which includes the

Box 2. The microtubule motors that drive mitochondrial motility

Mitochondria are actively shuttled within eukaryotic cells, localizing to regions of the cell with the highest energy demands. Mitochondria are bidirectionally transported along microtubules, with prolonged excursions towards the plus and minus ends of microtubules [57]. Motors from both the kinesin-1 and kinesin-3 families drive the plus end-directed transport of mitochondria, whereas cytoplasmic dynein is the major motor for transport toward the microtubule minus end [58] (Figure 1h).

Recent progress has revealed several adaptor proteins that link these motors to mitochondria. In *Drosophila*, the protein Milton interacts with kinesin and is required for the localization of mitochondria to axons [59]. Milton in turn interacts with the Rho-like GTPase Miro, which regulates the association of both Milton and kinesin with the mitochondria [25,26]. GRIF-1, an ortholog of Milton, might function similarly in mammalian cells to link kinesin-1 to mitochondria [60]. Alternatively, or possibly in addition to this mechanism, the protein syntabulin binds to kinesin-1 and co-localizes to mitochondria. Syntabulin is a linker protein that shares some homology to the $p150^{Glued}$ subunit of dynactin and binds to kinesin-1 and syntaxin, a key component of the membrane fusion machinery at neuronal synapses [61]. Disruption of syntabulin leads to impaired anterograde transport of mitochondria along neuronal processes [62].

The interaction of dynein with mitochondria is less well studied, but mutations in the protein APLIP1, a neuronally expressed scaffolding protein that binds JNK-family kinases, have been shown to disrupt the retrograde transport of mitochondria in *Drosophila* [63]. It is not yet clear whether APLIP1 interacts directly with dynein, or if the mechanism is indirect and involves signal transduction pathways.

Figure 1. Microtubule-based transport and intracellular trafficking pathways. Many intracellular trafficking pathways involve active and directed transport along the microtubule cytoskeleton. Microtubule-dependent trafficking in mammalian cells includes: **(a,b,c)** ER-to-Golgi transport, **(d)** TGN-to-ER transport and **(e)** lysosomal, **(f,g)** endosomal and **(h)** mitochondrial motility. The direction of organelle transport along the microtubule is denoted with black arrows (towards the MTOC is minus end-directed, away from the MTOC is plus end-directed). (a) COPII coats are concentrated at ER exit sites, and a subunit of the COPII coat has been shown to interact with dynactin, which could then act to recruit dynein and mediate microtubule minus end-directed motility of the ER-derived vesicle [30]. (b) ER-to-Golgi transport could be mediated by dynein through the binding of βIII spectrin to ARP1 subunit of dynactin [16,17]. (c) Dynein could also be recruited to ER-derived vesicles by ZW10, which exists in a complex with Rough deal, Zwilch and RINT-1. The ZW10 complex associates in turn with vesicles through an interaction with ER-associated protein syntaxin 18 [32–34]. (d) TGN-to-ER transport is mediated through an interaction of activated Rab6, present on TGN membranes, with BICD or BICD2 (BICD1/2). BICD then recruits dynein–dynactin to promote minus end-directed motility [22]. (e) Lysosomal minus end-directed movement is mediated through the Rab7 effector RILP, which in turn recruits dynein and dynactin to the lysosome [48]. The heterotrimeric kinesin-2 is a plus end-directed motor for late endosomes and lysosomes [43]. Other members of the kinesin superfamily might also contribute to lysosomal motility. (f) Plus end-directed motility of early endosomes purified from rat liver is dependent on kinesin-1, whereas minus end-directed motility has been shown to be dependent on KIFC2, a member of the kinesin-14B class. Rab4–GDP enhances this minus end-directed movement [41]. (g) Early endosomes purified from HeLa cells are transported to microtubule plus ends by the kinesin-3 family member KIF16B, which contains a PX motif in its tail domain that binds directly to phospholipids present on the endosomal membrane [21]. (h) Mitochondria are transported along the microtubules in the plus-end direction by both kinesin-1 and kinesin-3 and in the minus-end direction by dynein in *Drosophila* [58]. Similar motors are likely to drive mitochondrial transport in mammalian cells.

proteins ZW10 and RINT-1, interacts in turn with the ER-associated SNAP receptor (SNARE) protein syntaxin 18 [32–34]. These proteins, localized to ER exit sites through the interaction with syntaxin 18, could act in turn to recruit dynein and dynactin through a direct association between dynactin and ZW10 (Figure 1c). Knockdown of RINT-1 blocks ER-to-Golgi trafficking [34], and knockdown of either ZW10 [33] or dynactin [35] leads to Golgi dispersal, consistent with this model.

The Golgi

Both trafficking into and out of the Golgi and the integrity of the Golgi itself are dependent on microtubule motors (reviewed by Allan *et al.* [31]). Depolymerization of the microtubule cytoskeleton or disruption of dynein function results in the fragmentation of the Golgi [31]. Dynein can associate with Golgi membranes by several distinct mechanisms, including through Golgi-associated βIII spectrin [16,17] (Figure 1b) or ZW10 [33], as discussed earlier. A role for the small GTPase Cdc42 has also been proposed in regulating the recruitment of dynein to COPI-coated membranes [36]. Defining the relative contributions of these mechanisms to the overall regulation of Golgi dynamics will require further investigation.

The best example for a specific and regulated association of dynein with membranes is the role of the BicaudalD-related proteins BICD and BICD2, together with Rab6, in recruiting dynein to either the TGN or to transport vesicles moving from endosomes to the TGN (Figure 1d). BICD and its closely related isoform BICD2 are now considered to be golgins, a family of Golgi-associated proteins that share a common coiled-coil structure and are involved in tethering and structural support functions at the Golgi [37]. Studies on BICD and BICD2 have shown that these polypeptides bind to dynein–dynactin and to the activated form of Rab6 [22]. Activated Rab6 also binds directly to dynactin [23]. Thus, activation of Rab6 leads to the recruitment of BICD or BICD2 and dynactin, and this in turn leads to the recruitment of dynein [22].

Other links between golgins and motor-driven transport have been observed. Depletion of the golgin tGolgin-1 (also known as p230 or golgin-245) blocks trafficking from endosomes to the Golgi [38]. In *Drosophila*, an association between the golgin Lava lamp and dynein, dynactin and spectrin also suggests that golgins promote dynein-based motility of Golgi membranes [39].

The localization of the Golgi near the MTOC might depend on a dynamic balance between minus end-directed and plus end-directed motor activities. Depletion of kinesin-1 leads to a more compact Golgi structure, in contrast to the Golgi fragmentation observed upon depletion of dynein [31]. These observations are consistent with the idea that the localization and the organization of the Golgi are both dynamic.

Trafficking in the retrograde pathway from Golgi to the ER, such as the recycling of ER-resident proteins, is likely to be kinesin-dependent. However, the specific kinesins driving this process have not yet been firmly identified, probably because of functional redundancy [31]. Multiple kinesins also participate in the trafficking pathways that diverge from the TGN [31]. These pathways include transport to late endosomes and sorting to constitutive and regulatory secretory vesicles. Transport from the Golgi is often targeted to specific cellular domains, including the axon or dendrites of neurons and cell–cell junctions in epithelial cells (Box 3).

Box 3. Polarized delivery along microtubules

Polarized delivery from the TGN has a crucial role in neurons. The motors might mediate trafficking specificity, perhaps responding to directional cues from the microtubule cytoskeleton. For example, a tail-less construct of kinesin-1 that lacks a cargo-binding domain is targeted specifically to the axon, whereas a similar tail-less construct of kinesin-2 is targeted to both axons and dendrites [64]. Furthermore, motors might actively participate in establishing polarity. Imaging of axon specification during neuronal development indicates that tail-less kinesin-1 accumulates in a single neurite before axon specification and that the stable accumulation of this motor is one of the earliest markers for the axon [65].

Specialized trafficking with polarized delivery also has a role in non-neuronal cells, for example in the development of cell–cell contacts. The assembly of cadherin-mediated cell–cell interactions is dependent on both microtubules and microtubule motors. Cytoplasmic dynein localizes to the cell cortex where it tethers microtubule plus ends [66]. These microtubules serve as tracks for the kinesin-mediated transport of junctional components, such as N-cadherin [67,68] and p120catenin [69]. Disruption of microtubule tethering by inhibition of dynein slows the assembly of cell junctions (L.A. Ligon and E.L.F.H., unpublished). The directed trafficking of vesicles to the cleavage furrow during cell division could follow a similar mechanism [70].

Endosomal motility

Significant progress has been made on the mechanisms driving endosome motility, primarily as a result of the development of complementary cellular and *in vitro* assays. In the cell, the dynamics of GFP-labeled proteins, such as the early endosome marker Rab5, have demonstrated the long-range motility of endosomes along microtubules [40]. More recent, higher resolution studies on the movement of endocytosed quantum dots reveal both microtubule plus end-directed (~1 μm/s) and minus end-directed (~1.5 μm/s) motility with sufficient resolution to see single motor-driven steps of 8 nm, 16 nm and 24 nm along the microtubule lattice [10].

The motility of both early and late endosomes has been reconstituted *in vitro*; there are significant differences in the motors associated with each type of endosome, consistent with a regulated switch in motor recruitment. This selective recruitment of motors to cargo seems to be mediated either directly or indirectly by Rabs.

The *in vitro* motility of early endosomes, purified from HeLa cells, along microtubules is bidirectional and Rab5-dependent [40]. The kinesin-3 family member KIF16B transports endosomes to microtubule plus ends both *in vitro* and in cells (Figure 1g). KIF16B has a PX motif in its tail domain, which binds directly to PtdIns(3)*P* phospholipids. Localization of KIF16B to early endosomes is Rab5-dependent, but the mechanism is indirect. Rather than binding directly to Rab5, KIF16B is recruited through the association of its PX domain with PtdIns(3)*P*, generated by the localized stimulation of PtdIns 3-kinase by activated Rab5 [21].

The motility of early endosomes purified from rat liver has also been reconstituted in vitro [6]. These early

endosomes also move bidirectionally along microtubules. The plus end motility is kinesin-1-dependent but, surprisingly, the minus end-directed motility is not driven by dynein. Instead, minus end-directed motility seems to be driven by the kinesin-14B family member KIFC2 (Figure 1f). Unlike the studies in HeLa cells described above, the motility of these early endosomes *in vitro* is dependent on Rab4, rather than Rab5; it is not yet clear why there are differences in the specificity of the Rabs regulating similar processes in the two systems. The hydrolysis of GTP by Rab4 enhances minus end-directed motility and might also lead to enhanced endosomal fission, enabling segregation of receptors from ligands [41].

The motility of late endosomes is distinct from that of early endosomes because of the recruitment of a distinct set of motors. *In vitro*, late endosomes move primarily toward the minus end of microtubules, driven by cytoplasmic dynein [42]. The plus end-directed motor kinesin-2 is also associated with late endosomes [42,43]. Neither Rab5 nor Rab4 co-localizes with these vesicles; instead Rab7 is bound. This observation is consistent with a role for Rabs as specific regulators of motor protein recruitment.

Recently, an interesting new function for Rabs in regulating a switch from actin-based to microtubule-based motility has been proposed. Rab5 recruits the protein Huntingtin (Htt) to early endosomes through the Rab5-effector and Htt-binding protein HAP40. These endosomes interact preferentially with F-actin rather than microtubules [44]. Hydrolysis of Rab5-bound GTP leads to the dissociation of HAP40 and might signal a switch to microtubule-based transport. Following this switch, Htt remains bound and can then recruit other binding partners for microtubule-based motility, such as the Htt-binding protein HAP1, which binds directly to dynactin [45] and kinesin [46]. Switching from actin-based to microtubule-based motility would promote a switch from short-range to longer-range motility in the cell.

Lysosomal motility

Rab GTPases also have a role in regulating lysosomal motility. GTP-bound Rab7 binds to the effector protein Rab-interacting lysosomal protein (RILP) [47], which arrests Rab7 in the vesicle-bound, activated state and recruits both dynein and dynactin to the lysosome [48] (Figure 1e). RILP also recruits dynein–dynactin to phagosomes, where the motor complex promotes the formation of membrane extensions along microtubules leading to phagosomal maturation [49].

The effects of motor-driven transport on trafficking

Although the specific recruitment of motor proteins to vesicles and organelles is often regulated by trafficking proteins, such as Rabs, does the motor-driven transport of these membranes affect trafficking in turn? Here the data are more equivocal. Although studies *in vitro* suggest that motors are required for several trafficking steps, cellular studies in which motor expression is knocked down or motor activities are inhibited often show relatively mild perturbations in downstream trafficking events.

Inhibition of dynein–dynactin causes a dramatic alteration in the morphology of the Golgi but can have more subtle effects on trafficking into and out of this compartment. For example, overexpression of an inhibitory fragment of the dynactin subunit $p150^{Glued}$ only partially inhibits cargo export from the ER [30]. At the Golgi, overexpression of the dynamitin subunit of dynactin partially inhibits Rab6-regulated recycling back to the ER [50].

In endosomes, overexpression or knockdown of the endosomal kinesin-3 KIF16B alters the localization of organelles within the cell, but the effects on trafficking are limited. Only minor changes in the balance between receptor recycling and degradation have been observed [21]. In all these cases, the partial inhibition of trafficking observed could reflect either incomplete knockdown or inhibition, or functional redundancy among the microtubule motors.

Alternatively, the subtle effects on trafficking observed upon knockdown or inhibition of individual motors might not be surprising, given the ability of cells to effectively traffic molecules through the ER and Golgi in the complete absence of microtubules [51]. In a mammalian cells growing in culture, microtubule-based transport could be dispensable for many trafficking pathways. For example, cultured neurons remain healthy for days in the absence of measurable levels of cytoplasmic dynein [52].

Actin-based motors might compensate for loss of microtubule motor activity or, in cells growing in culture, motor-driven processes might function only to enhance trafficking efficiency. For example, membrane fusion could be enhanced by the action of motors driving vesicles close enough to fuse with higher frequency. Membrane fission might be enhanced through motor-driven membrane extensions along the microtubule.

By contrast, intracellular transport processes appear to be significantly more important in the context of an intact organism. The complex interplay of multiple cell types during development, or the significantly longer cellular processes that must be supported *in vivo*, seem to require highly efficient and highly polarized transport. Thus, the effects of motor protein dysfunction on intracellular trafficking might become more apparent *in vivo* [35].

Conclusion: progress at the intersection of trafficking and transport

Recent progress in our understanding of motor–cargo interactions has provided mechanistic insights into the links between trafficking and transport. We can now begin to understand what is transported where and when within the cell. The challenge is now to understand how trafficking and transport are coordinately regulated, both temporally and spatially. The involvement of Rabs and other GTPases in regulating the recruitment of motors to membranes will serve as a paradigm for understanding some types of cellular transport. However, there does not seem to be a conserved mechanism for Rab-mediated motor recruitment. In some cases the recruitment is mediated by protein–protein interactions and in other cases by alterations in membrane lipids mediated by effectors downstream of Rabs. Other transport processes are likely to be differentially regulated. Motor recruitment to some organelles is mediated by scaffolding, adaptor, or coat proteins. These mechanisms also enable temporal and spatial modulations of motor association.

Once bound to cargo, the activity of the motor is likely to be further regulated. Oppositely oriented motors have been observed to associate stably with cargo during bidirectional transport, rather than cycling on and off the membrane [7]. This suggests that there are cellular regulatory mechanisms that function to turn on or turn off the activity of the bound motor proteins. However, little is yet known about the mechanisms that modulate the activity of cargo-bound motors.

In summary, progress has been rapid in recent years in our understanding of the functional specification of the motors that drive intracellular trafficking, and in the identification of mechanisms that regulate motor recruitment to intracellular membranes. This work has identified some common themes and a significant degree of diversity in mechanisms of motor recruitment and cargo transport. Taken together, however, this progress raises the expectation that we might soon have a trunk-to-tail understanding of trafficking and transport within the cell.

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