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Functional coupling of microtubules to membranes: implications for membrane structure and dynamics

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Summary

The microtubule network dictates much of the spatial patterning of the cytoplasm, and the coupling of microtubules to membranes controls the structure and positioning of organelles and directs membrane trafficking between them. The connection between membranes and the microtubule cytoskeleton and the way in which organelles are shaped and moved by cytoskeletal interactions have been studied intensively in recent years. Particularly, recent work has expanded our thinking of this topic to include the mechanisms by which membranes are shaped by coupling to the cytoskeleton and by which cargo selection is directed, both through the formation of transport carriers and the nature of the directed movement of these carriers themselves. In this Commentary, I will discuss the molecular basis for membrane–motor coupling and the physiological outcomes of this coupling, including the way in which microtubule-based motors affect membrane structure, cargo sorting, and vectorial trafficking between organelles. Whereas many core concepts of these processes are now well understood, key questions remain about how the coupling of motors to membranes is established and controlled, about the regulation of cargo and/or motor loading, and about the control of directionality.

Introduction

Cellular function is underpinned by the spatial organization of intracellular organelles. Despite our growing understanding of various organelle functions, many questions remain as to why cells are organized in the way they are. These include the reasons for maintaining a juxtanuclear Golgi network, a highly distributed but dynamic endoplasmic reticulum (ER), or specifically positioned endosomal compartments. We do know, however, that this organization is largely governed by the cytoskeleton. The microtubule and actin networks both have substantial roles in membrane organization and trafficking and are used to differing extents by different organisms. *Saccharomyces cerevisiae*, from which we obtained much of our knowledge of the core membrane trafficking machinery, relies almost exclusively on the actin network for membrane trafficking. Plants similarly use actin as the major cytoskeletal element. Our knowledge of the role of actin has exploded recently with the identification of many new regulators of both actin–membrane coupling and actin nucleation at different organelles. This has been discussed in a very informative recent review (Anitei and Hoflack, 2011). In this Commentary, I will, therefore, exclusively deal with the microtubule network and its importance in endomembrane organization and trafficking in animal cells. For example, the depolymerization of microtubules disperses the Golgi apparatus (Sandoval et al., 1984), perturbs the dynamics of the ER (Terasaki et al., 1986; Foissner et al., 2009), and affects the positioning of endosomal and lysosomal compartments (Matteoni and Kreis, 1987). In many cases we have begun to define the molecular basis for the physical link between microtubules and these membranous organelles, and microtubule-based motor proteins are of central importance in this process.

Both microtubule motors, kinesin and dynein, convert the energy from ATP into force that drives their translocation along microtubules. Nearly all kinesins are plus-end directed, although there are some notable exceptions. Cytoplasmic dynein-1 is the primary minus–end-directed motor and is involved directly in intracellular membrane dynamics. In the context of this Commentary, the term ‘dynein’ will thus be used to refer to cytoplasmic dynein-1. The ~45 members of the kinesin superfamily and the composition of the dynein motor have been described in detail in recent reviews (Hirokawa et al., 2009) (Allan, 2011). All of these motors can, of course, move many different cargoes, but membrane-bound vesicles, tubules, and indeed entire organelles form a key part of this cohort (Figure 1).

Microtubule organization and motor function are intricately linked with multiple membrane trafficking pathways. ER-to-Golgi transport, the organization of the Golgi, and the positioning of (and trafficking within) the endo-lysosomal systems are processes that exploit motor proteins (Figure 1). In all cases, motors are known to modulate organelle structure and position, as well as to drive the vectorial transfer of cargo between compartments (Hirokawa et al., 2009; Allan, 2011). More recently, we have also gained further understanding of the role of specific membranes (such as those of the Golgi) in microtubule nucleation. The motility of organelles and transport carriers is the most obvious outcome of membrane–microtubule coupling, but one must also consider the impact of the cytoskeleton on the formation of transport vesicles as well as the shape and steady-state location of organelles. The fundamental importance of motors in other cellular processes, such as mitosis, has been demonstrated clearly, and these will not be discussed in any depth here. In this Commentary, I will discuss our current state of knowledge regarding the molecular mechanisms used by the microtubule network and its associated proteins to shape and traffic membranes. I will

focus, in particular, on the secretory and endocytic pathways but many of themes discussed are common to the coupling of microtubules to other membranes such as those of the nucleus and mitochondria. Other recent reviews provide an excellent discussion of these topics (Boldogh and Pon, 2007; Starr, 2011). I will include a discussion of the numbers of motors that are required for these processes, the mechanism by which these motors are functionally coupled to membranes, and the implications for the morphology of membranes, intracellular organization, and membrane traffic. In addition, I will highlight our growing understanding of the mechanisms used by membranes to generate specialized microtubule networks by focusing on nucleation at the Golgi apparatus as an example.

Numbers and types of motors associated with dynamic membranes

The cytoplasm is an incredibly dynamic environment, and the organelles within it portray complex dynamics (Figure 1). A major question with regards to motor-protein driven organelle motility is how many individual motors of one type are required to move a specific cargo. However, analyses of the activity of motor proteins and their coupling to cargo are complicated by the very small number of motors that are required at any one time (Shubeita et al., 2008; Hendricks et al., 2010; Schuster et al., 2011). This makes detection of motors by imaging or biochemistry difficult. This problem has been addressed in recent years by making use of developments in single molecule imaging (Veigel and Schmidt, 2011) and through the elegant exploitation of unusual model systems such as the filamentous fungus *Ustilago maydis* (Steinberg and Perez-Martin, 2008). Strong evidence now implicates as little as one motor in generating the motility of individual organelles in vitro and in vivo. For example, studies investigating the movement of Lysotracker™-positive organelles in neurons (Hendricks et al., 2010) and using optical tweezers to accurately measure stall forces of motors that are attached to lipid droplets in *Drosophila* embryos have shown that a single motor protein is able to move an organelle (Shubeita et al., 2008). It is worth noting, however, that in the case of lipid droplets, more than one motor are typically associated with each organelle at any one time. The physiological reasons for this remain unclear, because this increase in number increases neither the speed of the organelle nor the distance moved (Shubeita et al., 2008). The association of a single motor is not only sufficient for the movement of early endosomes in *Ustaligo maydis*, but can also cause a change in the direction of travel (Schuster et al., 2011). An elegant combination of in-vitro and in-vivo work (using *Dictyostelium*) has shown that only 4-8 weakly attached dyneins and a single stronger kinesin drive motility of early endosomes (Soppina et al., 2009).

Both kinesins and dynein can be attached to the same cargo at the same time (Shubeita et al., 2008; Soppina et al., 2009; Hendricks et al., 2010). The opposing forces generated by plus-end directed kinesins and minus-end directed dynein provide the opportunity for bidirectional motility along microtubule filaments. Bidirectional motility has been observed for mitochondria (Morris and Hollenbeck, 1993), pigment granules (Rogers et al., 1997), secretory vesicles (Matanis et al., 2002; Grigoriev et al., 2007), the ER (Wozniak et al., 2009), and endocytic vesicles (Murray et al., 2000; Soppina et al., 2009) and the mechanisms underlying the coupling of opposing motors has received considerable focus in recent years (see Box 1).

Coupling of microtubule motors to membranes

The identification of the kinesin and cytoplasmic dynein motors (Vale et al., 1985a; Vale et al., 1985b; Paschal et al., 1987; Vallee et al., 1988) and the defining experiments validating their role in intracellular membrane motility (Schnapp and Reese, 1989; Schroer et al., 1989) have transformed our views of membrane dynamics. The identification of the dynactin complex (Gill et al., 1991) and the role of the $p150^{Glued}$ dynactin subunit in linking dynein to dynactin(Vaughan and Vallee, 1995; Waterman-Storer et al., 1995), as well as the proposal that dynactin acts as a direct adaptor that links dynein to membranous cargo [summarised in (Allan, 2000)] have been pivotal to many of the discoveries that followed.

Motors can associate with cargo by either direct or indirect mechanisms. In many cases, even though the motor subunit that is necessary for the interaction has been identified, it remains unclear whether cargo binds directly to the motor. Indeed, in most cases accessory factors seem to be required. The following sections focus on the evidence for direct and indirect coupling of motors to membranes.

Direct binding of motors to membrane cargo

Kinesin and dynein must employ different mechanisms to couple to membranes, because the kinesin superfamily includes a large number of motor subunits, many of which bind accessory light chains to define their function (Hirokawa et al., 2009), whereas dynein is built around a single motor subunit whose functional specialization is provided by multiple additional subunits (Allan, 2011).

With regards to kinesin, it has been shown that different kinesin light chains exhibit specificity for ER and Golgi-derived membranes in an in vitro assay (Wozniak and Allan, 2006) and in live cells (Wozniak et al., 2009). These finding supports the concept that kinesin light chains specify cargo interactions. However, no direct link between kinesins and ER membranes has been identified so far. More recently kinesin light chain (KLC) 2 has been found to be selectively involved in Na+/K+-ATPase trafficking to the plasma membrane (Trejo et al., 2010) and KLC1 appears to mediate calsyntenin trafficking (Vagnoni et al., 2011). In this case, binding between the light chain and calsyntenin is direct and the motif in KLC1 that is responsible for this has been mapped (Konecna et al., 2006). This protein interaction motif is conserved in the Vaccinia virus protein A36R, which has led to the elucidation of a kinesin-1 binding signature in many proteins (Dodding et al., 2011; Dodding and Way, 2011). Notably this includes many membrane proteins, as well as the dynein intermediate chain, which had previously been implicated in binding to KLC1 (Ligon et al., 2004). The direct interaction between kinesin and dynein provides a potential mechanism for direct coupling of opposing motors.

Kinesin family member (KIF) 16B [also called sorting nexin (SNX) 23] provides an example of a motor that can couple directly to membranes by virtue of a phosphoinositide-binding phox-homology (PX)-domain in its carboxyl-terminus (Hoepfner et al., 2005). Dual sensing of the membrane through its lipid content and through a Rab-GTPase (in this case GTP-bound Rab14) by KIF16B provides an example of coincidence detection, meaning that both Rab14 and phosphoinositide binding are required for KIF16B recruitment. This ensures targeting of KIF16B to the correct membrane at the correct point of the vesicle transport cycle (Ueno et al., 2011). Rab5 might have a role in parallel with Rab14, but a direct interaction between Rab5 and KIF16B has not been demonstrated (Hoepfner et al., 2005). The Unc-104 (KIF1A) kinesin-3 family motor binds directly to phosphatidylinositol-4,5-

bisphosphate (PtdIns(4,5)P_2 in vitro (Klopfenstein et al., 2002) and in vivo (Klopfenstein and Vale, 2004). This motor is involved in the transport of cargo along neurons to synapses, and some recent genetic data suggest that, when not bound to cargo, the motor is in fact degraded (Kumar et al., 2010). Other data suggest that coincidence detection is also crucial for KIF1A function since both SYD-2 (for sunday driver-2, also known as MAPK8IP3) (Wagner et al., 2009) and PTL-1 (protein with tau-like repeats, the *C. elegans* homologue of tau) (Tien et al., 2011) regulate recruitment and/or activity of this motor.

The function of dynein heavy chains is regulated by its association with mutually exclusive additional subunits. The dynein light intermediate chain 1 (LIC1) has a major role in Golgi function, whereas LIC2 has a more central role in the recycling of endosomes (Palmer et al., 2009). However, other studies suggest that both LICs operate redundantly (Sivaram et al., 2009; Allan, 2011; Tan et al., 2011), and some studies have failed to find any role for LICs in Golgi maintenance (Sivaram et al., 2009; Tan et al., 2011). Comparison between these experiments is, of course, complicated by variability in experimental set-ups such as the efficiency of siRNA depletion and subtle differences in assay readout. It is also possible that cell-type-specific differences explain these discrepancies, at least in part. However, none of these studies define whether LICs mediate direct or indirect coupling between motors and membranous cargo, leaving many questions to be answered in future studies.

Indirect coupling of motors to membrane cargo

In most cases, the coupling between motors and membranes has been found to involve intermediate factors. Foremost among these is the dynactin complex described above (Kardon and Vale, 2009). The inhibition of the interaction between dynein and dynactin affects multiple cellular trafficking events, including ER–Golgi trafficking, Golgi structure, and endosomal function (Burkhardt et al., 1997; Presley et al., 1997). There has, however, been some disagreement with regards to the nature of dynactin function in this context. Dynactin contains two microtubule binding sites within its p150 subunit (Culver-Hanlon et al., 2006) and has direct effects on the function of dynein [such as, for example, increasing its processivity (King and Schroer, 2000)]. However, the removal of the microtubule-binding domain of p150 has no effect on Golgi organization (Dixit et al., 2008) or membrane transport in *Drosophila* S2 cells (Kim et al., 2007). Other work has suggested that dynactin is not essential for the targeting of dynein to membranes (Kumar et al., 2001; Haghnia et al., 2007; Flores-Rodriguez et al., 2011). By contrast, a recent study using *Aspergillus nidulans* showed quite convincingly that the p25 subunit of dynactin – which is not required for the integrity of the dynactin complex or for the association of dynactin with dynein – is required for targeting the motor complex to early endosomes (Zhang et al., 2011). This discrepancy is perhaps explained by differences between species, or even cell-types. The ability of dynactin to bind microtubules is most likely required in situations where greater force is required, such as during microtubule organization (Kim et al., 2007) or nuclear migration (Kardon et al., 2009; Starr, 2011).

Multiple other accessory factors have been implicated in dynein recruitment, many of which probably act in concert to couple membrane deformation, vesicle formation, and, possibly, the capture of cargo with microtubule-based motility (Kardon and Vale, 2009). Key examples include the integration of Rab GTPase function with motor activity. Rab4, Rab5, Rab6, Rab7 and Rab11 are key examples of this and the direct association of Rabs with motors has been demonstrated. For example, members of the Rab6 family bind directly to the dynein light chain roadblock-type 1 (DYNLRB1) (Wanschers et al., 2008) and Rab4a has been shown to bind directly to dynein light

intermediate chain 1 (DYNC1LI1) (Bielli et al., 2001). In many cases Rabs act in concert with other dynein binding proteins such as bicaudal D homologue (BICD), which acts with Rab6, or RAB11 family interacting protein 3 (RAB11FIP3), which acts with Rab11. For further details on the relationship between Rabs and motors, I refer the readers to three recent reviews (Allan, 2011; Horgan and McCaffrey, 2011; Hunt and Stephens, 2011).

The BICD family is of particular interest with regards to membrane dynamics at the Golgi. They are now considered to be part of the golgin protein family (Barr and Short, 2003) and recruit and regulate dynein to direct traffic at the *trans* face of the Golgi complex (Matanis et al., 2002). Rab6 can recruit BICD family proteins, but also can recruit dynactin (Short et al., 2002). Furthermore, BICD2 can recruit the dynein–dynactin complex (Hoogenraad et al., 2001). The N-terminal domain of BICD2 can recruit dynein in the absence of accessory factors (Hoogenraad et al., 2003), which suggests that the role of this complex series of interactions between Rab6, BICD, dynein and dynactin must relate to spatial and/or temporal control of minus-end directed traffic around the Golgi. The plethora of dynein-interaction sites on BICD and its associated molecules, together with the fact that the coiled-coil domains of BICD likely mediate its oligomerization, leads to the possibility that it acts in assembly of larger multi-motor assemblies [reviewed in (Dienstbier and Li, 2009)]. In addition to binding to its role in recruiting dynein to membraneour organelles, BICD has also been shown to bind kinesin with low affinity (Grigoriev et al., 2007).

Increasingly, evidence for the functional integration of motor regulation and cargo binding is being uncovered (Kardon and Vale, 2009). A clear example of this comes from the nuclear distribution protein E (NDE1) and NDE1-like proteins. These two proteins appear to act redundantly with regards to membrane motility (Lam et al., 2010). Intriguingly they not only affect dynein activity, but they are also required for maintaining the association of dynein with membranes (Lam et al., 2010). NDE1 and Nudel (also known as NDEL1) both act in the recruitment of LIS1 (for lissencephaly-1) to membranes (Liang et al., 2004; Lam et al., 2010). LIS1 can itself bind to dynein (Faulkner et al., 2000; Smith et al., 2000), inducing a “persistent force” state thought to be required for dynein to move larger cargo (McKenney et al., 2010). The role of LIS1 in targeting dynein to membranes is not entirely clear. LIS1 is also a component of the phospholipase platelet-activating factor acetyl hydrolase 1b (PAFAH1B), which modulates membrane structure and dynamics (Bechler et al., 2010; Bechler et al., 2011). Most likely phospholipid remodelling and motor function are both involved in LIS1-dependent membrane dynamics. An interesting additional aspect of LIS1 function comes from the recent finding that cAMP-specific phosphodiesterase 4 (PDE4) can negatively regulate the association of LIS1 with dynein, thereby providing a potential hub that integrates signalling with LIS1-dependent dynein function (Murdoch et al., 2011). Thus, NDE1 and LIS1 appear to act together to generate a dynein complex that is capable of moving heavy loads, whereas dynactin acts to enhance processivity. This is strongly supported by the fact that LIS1 and dynactin appear to bind in a mutually exclusive manner to dynein (McKenney et al., 2011).

In summary, direct coupling of motors to membranes would have the advantage of simplicity in controlling membrane dynamics, whereas indirect mechanisms using one or several additional adaptor(s) provide more scope for integrating force generation with other cellular activities such as activation of Rabs. Whether direct or indirect, control of the interaction between motors and membranes through different means is a key mechanism in the regulation of membrane dynamics. An example of how this interaction can be dynamically affected is the regulation of the association

between motors and their cargoes by protein phosphorylation (Yeh et al., 2006; Guillaud et al., 2008). Similarly, phosphorylation of the dynein LIC1 by cyclin-dependent kinase 1 (CDK1) leads to the dissociation of the light chain from the membrane (Niclas et al., 1996; Addinall et al., 2001).

Roles for motors in membrane remodelling

It has been known for some time that motors are involved in shaping the ER network and driving its motility (Terasaki et al., 1986; Vale and Hotani, 1988). Early reconstitution events demonstrated the ability of motors to drive the formation of intricate membrane networks in vitro (Vale and Hotani, 1988; Allan and Vale, 1994). Both kinesin and dynein are relevant in this process, and indeed this is also true in live cells (Wozniak et al., 2009). The physiological relevance of a dynamic ER remains unclear. Possible functions include roles in development (Lane and Allan, 1999), Ca^{2+} signalling [for example in terms of ER–plasma membrane coupling for capacitative calcium entry (Grigoriev et al., 2008; Orci et al., 2009)], spatial organization of protein synthesis or trafficking, or perhaps in metabolic sensing [through coupling of the ER to the mitochondrial network (Friedman et al., 2010)]. In addition, the link between ER and mitochondrial dynamics is an area of great interest at present, because the ER defines sites of mitochondrial fission (Friedman et al., 2011) and mitochondrial morphology is directly linked to autophagy (Gomes and Scorrano, 2008; Gomes et al., 2011; Rambold et al., 2011).

The tubular nature of many other organelles such as endosomes has been clear for some time (Hopkins et al., 1990). In some cases, tubules can be generated and maintained by proteins such as the sorting nexin family members containing BAR domains (SNX–BAR family) (van Weering et al., 2010). However, considerable evidence also implicates the microtubule network in tubule structure and function. Elegant in vitro reconstitution studies have shown that artificially coupling microtubule motors to synthetic liposome membranes can generate tubules (Roux et al., 2002). Tubule-generating SNX–BAR family members have been found to interact with the dynein–dynactin motor complex either directly or indirectly, thereby coupling the membrane sculpting activity of the SNX–BAR proteins to the application of force by motor proteins. Examples of such interaction include the binding of the retromer components SNX5 and SNX6 to the p150 dynactin subunit during endosome to trans-Golgi network (TGN) trafficking (Hong et al., 2009; Wassmer et al., 2009), and the interaction of SNX4 with dynein through KIBRA (kidney- and brain-expressed protein, also known as WWC1) during endosomal recycling (Traer et al., 2007). The ability of motor proteins to impart force on membranes leads to two obvious mechanistic implications for membrane dynamics (Figure 2). First, the force that is generated by motors that are coupled to membranes can shape organelles (Fig. 2A, C). Second, the application of longitudinal force has consequences for membrane scission (Fig. 2A, B (Hong et al., 2009; Wassmer et al., 2009)). Third, coupling of motors to cargo selection machineries could drive cargo segregation into discrete domains (Fig. 2D). The work of Soppina et al (2009) provides an elegant example of the major concepts shown in Fig. 2. Opposing motors drive bidirectional motility of endosomes coupled to deformation and indeed fission (Soppina et al., 2009). Whereas this study did not provide any evidence of cargo sorting, it does support the idea that the opposing motors segregate to distinct domains of the organelle; if coupled indirectly to cargo molecules, this would result in concomitant cargo segregation (as illustrated in Fig. 2D). Alternatively, tubulation might facilitate geometric cargo sorting (i.e. the partitioning of cargo into the tubular domain of the endosome, see Fig. 2C).

It seems likely that many membrane budding events use motor coupling as a mechanism to drive tubulation and/or scission. Indeed, motors and their accessory proteins can also couple directly to vesicle coat complexes. Examples of this include the binding of KIF13A to the AP1 clathrin adaptor (Nakagawa et al., 2000), the recruitment of the dynein–dynactin complex to the COPII (for coat protein complex II) coat during ER export (Watson et al., 2005) and to COPI at the Golgi (Chen et al., 2005), as well as recruitment of the dynein–dynactin complex to the SNX5/SNX6-containing retromer complex discussed above (Hong et al., 2009; Wassmer et al., 2009).

Motors in organelle positioning and signal transduction

The endo-lysosomal system is central to metabolic sensing by being involved in the trafficking of signalling complexes. Receptor trafficking through this system is relevant to the localization of active signalling complexes, but also impacts on the duration of signals by redirecting receptors to the recycling pathway or towards degradation. Similar concepts underpin regulated trafficking of adhesion molecules and plasma membrane ion channels and transporters. Endo-lysosomal positioning has been shown to regulate complex physiological outcomes, such as decoding of morphogen gradients during development [see (Rainero and Norman, 2011)].

In addition to mediating the coupling of motors to membranes during membrane trafficking as discussed above, it appears that Rabs have a central role in organelle positioning. Rab7 interacts with dynein–dynactin through a series of adaptors including Rab-interacting lysosomal protein (RILP) (Jordens et al., 2001). RILP interacts directly with Rab7 and dynactin, yet this complex is not sufficient to drive endosome motility. Two other factors, the oxysterol-binding protein related protein 1L (ORP1L) and beta-III spectrin [which acts as a general receptor for dynactin on membranes (Holleran et al., 2001; Muresan et al., 2001)], are also required (Johansson et al., 2007). It has been proposed that ORP1L senses the cholesterol status of the late endosomal membrane and can direct peripheral, low cholesterol-containing Rab7-positive late endosomes to interact with the ER by binding to vesicle-associated protein (VAP) (Rocha et al., 2009). This removes the dynein–dynactin components, allowing plus–end-directed transport of late endosomes. This role for Rab7 gives insight into the coupling of metabolic sensing with motor activity to regulate organelle positioning, and the same interaction network has also been implicated in the positioning of secretory granules in cytotoxic T lymphocytes (Daniele et al., 2011).

Modulation of organelle position has direct effects on cellular metabolism. Again this is particularly evident for lysosomes. Metabolic flux mediated through mTOR (mammalian target of rapamycin) signalling is directly linked to lysosomal function. Signalling through the mTOR complex 1 (mTORC1) is both activated and terminated by lysosomes (Sancak et al., 2010). Recent data have linked mTORC1 signalling to autophagy (Ravikumar et al., 2009), where nutrient availability modulates lysosome positioning such that, at times of low nutrient availability, lysosomes are clustered in the cell centre (Korolchuk et al., 2011). This appears to be regulated by pH (Heuser, 1989), but the mechanisms behind this remains unclear. Of particular relevance to the topic of this Commentary is the fact that the position of lysosomes appears to be correlated with the rate of autophagosome–lysosome fusion (Korolchuk et al., 2011), which in itself is a key regulator of autophagic flux. Dynein is known to enhance the efficiency of autophagosome–lysosome fusion (Kimura et al., 2008),thus the balance of bidirectional motility is highly likely to be a key determinant of autophagic flux.

Microtubule dynamics drive membrane movement

Whereas directed translocation of membrane-bound vesicles, tubules, and organelles by motor proteins is the most common mechanism for microtubule-based movement, one must also consider the motility of microtubules themselves. Static links between organelles and microtubules provides another means for directed movement (see Figure 1A). The concept of microtubule sliding is not new and, indeed, an extensive literature describes this with respect to translocations of microtubules within neurites and during the extension processes of these cellular structures [see (Cleveland and Hoffman, 1991)]. Microtubule sliding is central to the organization of the mitotic spindle and, here, might have a role in organelle partitioning during mitosis [for example see (Goshima et al., 2005)]. The impact of motor force on microtubule structure would also have indirect consequences for any attached organelle (Bicek et al., 2009). A further example is provided by the attachment of the ER to microtubule tips, which results in the extension of the ER in response to microtubule polymerization (Grigoriev et al., 2008). The important point here is that static links can result in changes in organelle shape and position without any direct coupling of a motor to that membrane.

Nucleation of microtubules by membranes and implications for directed transport

In addition to the impact of pre-existing microtubules on membranes, in recent years we have gained considerable knowledge into the molecular mechanisms that govern the way in which membranes modulate the structure of the microtubule network. This is nicely illustrated by the by the ability of Golgi membranes to nucleate microtubules in epithelial cells [(Chabin-Brion et al., 2001) and see Figure 3A]. Intriguingly, this appears to be controlled by a CLASP2- (for cytoplasmic-linker-associated protein 2) and GCC185- (for Golgi coiled coil protein of 185 kD) dependent mechanism at the trans face of the Golgi (Efimov et al., 2007). By contrast, a GM130- (for Golgi matrix protein of 130 kD) and AKAP450- (for A-kinase anchoring protein of 450 kD) dependent mechanism controls microtubule nucleation at the cis-face of the Golgi (Rivero et al., 2009). AKAP450 (also called CG-NAP, centrosome and Golgi localized protein kinase N-associated protein, or hyperion) also anchors microtubules at the centrosome (Takahashi et al., 2002), which suggests that it could act as a general microtubule nucleator. The precise relationship between these two mechanisms (if any) remains to be clarified. Given that Golgi cisternae are highly dynamic, and change their biochemical properties as they mature, one might consider that microtubules nucleated at the *cis*-side of the Golgi by (Glick and Nakano, 2009) GM130 remain attached through a CLASP2-dependent mechanism before taking on a distinct GCC185-dependent function at the *trans*-face.

We are also beginning to understand the cellular functions of membrane nucleated microtubules. Nucleation of microtubules by the Golgi provides the cell with non-centrosomal systems for targeted delivery of cargo to and from this organelle (Miller et al., 2009). These protein interaction networks based around CLASP2 also appear to be related to the functional coupling of the Golgi and the centrosome during processes such as their coupled relocalisation to the front of migrating epithelial cells (Hurtado et al., 2011). The TGN-nucleated microtubule array is aligned with septin filaments (Spiliotis et al., 2008) and ultimately forms a post-translationally modified set of microtubules that is generated by septins, which can act directly on microtubule dynamics (Bowen et al., 2011). At least in some cells, this network has a role in the targeted delivery of cargo to the plasma membrane (Schmoranzer et al., 2003; Dunn et al., 2008; Yadav et al., 2009). It has been shown that kinesin-1

exhibits a preference for such modified tracks (Reed et al., 2006; Dunn et al., 2008; Hammond et al., 2010). Post-translational modification of tubulin also regulates the interplay between microtubules and the intermediate filament network, which could, of course, also have key implications for membrane dynamics (Kreitzer et al., 1999). The functional interplay between microtubule, actin, intermediate filament, and septin filament networks is likely to be of great significance in vivo.

Perspectives

This Commentary highlights some of the key features that govern our understanding of the coupling between membranes and microtubules. Although I have largely focussed on the role of microtubule motors, it is increasing clear that the relationship between membranes and microtubules themselves is increasingly important with regard to intracellular organization and organelle function. Examples include the sliding of microtubules against one another and the role of membranes in the direct nucleation of new microtubules. The direct relevance of motor function to developmental processes underscores the importance of gaining a full understanding of the mechanistic basis for functional coupling of the endomembrane network to the cytoskeleton. Rab14-dependent coupling of KIF16B to FGF-receptor vesicles (Ueno et al., 2011) and the role of kinesin-3 and dynein in neural stem cell migration (Tsai et al., 2010) are both good examples of this. Mutations in motors and their accessory proteins also lead directly to a variety of diseases, notably those affecting the brain. Some mutations are attributed to changes in dynein activity [for example, mutations that change the processivity of dynein, e.g. (Hafezparast et al., 2003; Ori-McKenney et al., 2010)]. In addition, mutations in dynactin have been linked to motor neuron disease (Puls et al., 2003).

Motor proteins remain at the core of any discussion of membrane dynamics and their roles not only in directed movement of transport vesicles and tubules but also in shaping membranes and driving the formation of these membrane trafficking carriers has become clear. However, the analysis of the role of motors in membrane dynamics is complicated by several factors. The small numbers of molecules involved in the organization and movement of membranes, coupled with mechanisms such as coincidence detection (where two components, e.g. a protein and phosphoinositide, are required simultaneously to specify membrane localization) present great challenges to the identification of relevant molecular machinery. The future probably lies in the integration of in-vivo and in-vitro approaches, as well as incorporation of mathematical modelling and computational data analysis. We are beginning to get the sense that one cannot translate from one experimental system directly to another: what happens in flies is not necessarily conserved in mammals and vice versa. Furthermore, it is quite apparent how little we know, not only of the mechanics, but also of the physiological relevance of membrane microtubule coupling in the context of tissues and, indeed, whole organisms. Clearly there is still a long way for these motors to go.

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Box 1: Bidirectional motility

In vivo, organelles do not move with a simple linear trajectory from A to B. Their path is interspersed with many stops and starts and, indeed, frequent changes of direction. The saltatory ('stop–start') motility could relate to the competing activity of opposing motors (i.e. a 'tug-of-war' between those motors generating force towards the minus or plus ends, respectively) (Soppina et al., 2009). Alternatively, there are examples where such rapid changes of direction are triggered by the specific recruitment of opposing motors (Schuster et al., 2011). The physiological reasons for opposing motors acting on the same cargo is not entirely clear, but possible reasons for such a set-up could be to avoid 'roadblocks' (i.e. the congestion of microtubule tracks by cargo), to optimize the coupling of motors to their cargo (through search-and-capture type mechanisms), to proofread the direction of travel, or to facilitate interactions between cargoes [as discussed in a recent review by (Jolly and Gelfand, 2011)]. At least in the case of peroxisome motility in *Drosophila* S2 cells, opposing motors are absolutely required (Ally et al., 2009), and depletion of either the relevant plus-end directed or minus-end directed motor halts motility. Incorporation of any of a diverse number of opposing motors sufficed to reinitiate peroxisome motion, as long as two opposing motors were present (Ally et al., 2009). However, opposing motors are not always required, and there are many examples where inhibition of one motor does not inhibit transport, which include motility of the ER network (Wozniak et al., 2009) and of endocytic organelles (Caviston et al., 2007).

The complexity resulting from the involvement of two types of motors in cargo trafficking raises the question about whether motors are not physically coupled to each other at all (panel A in figure), are coupled directly (panel B in figure), or are linked through a common linker that could act as a common point of regulation (panel C in figure). Candidates for the regulation of opposing motors by a single adaptor include huntingtin (Colin et al., 2008) and the JNK family of interacting proteins (JIPs) (Montagnac et al., 2009). Analysis of the morphology of endosomes has suggested that opposing motors likely act on distinct domains of one organelle during its translocation (Soppina et al., 2009). This is further supported by the multitude of coupling factors that exist on the same organelle (for example SNX1 and SNX4 colocalize on early endosomes but couple to dynein by distinct mechanisms that specify discrete trafficking pathways (Traer et al., 2007; Wassmer et al., 2009). In addition, the notion that unregulated competition dictates trafficking directionality is supported by mathematical modelling (Muller et al., 2008).

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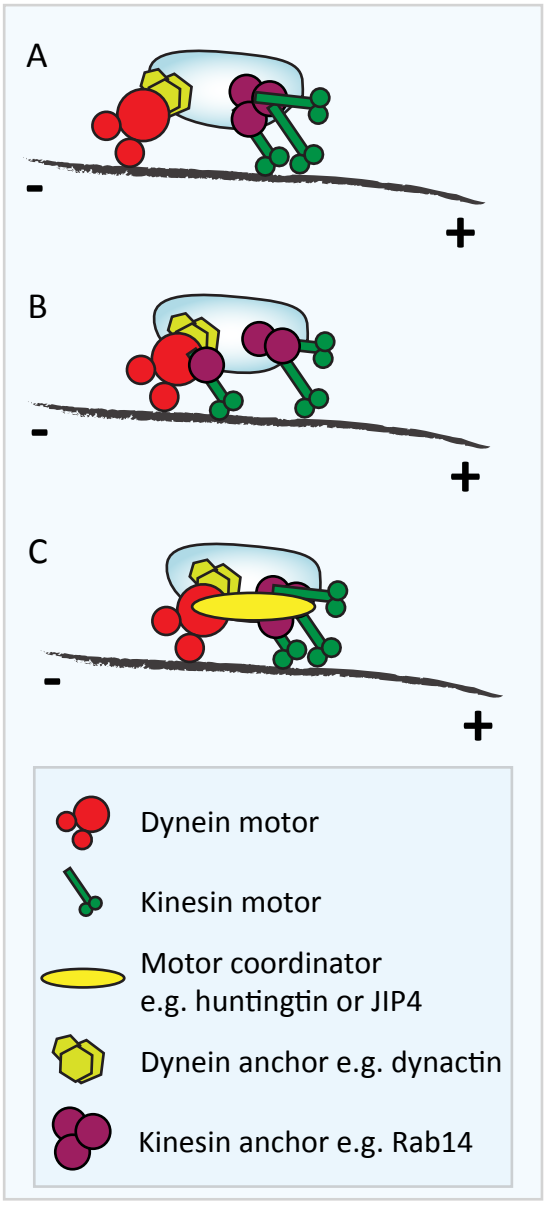
Figure legends:

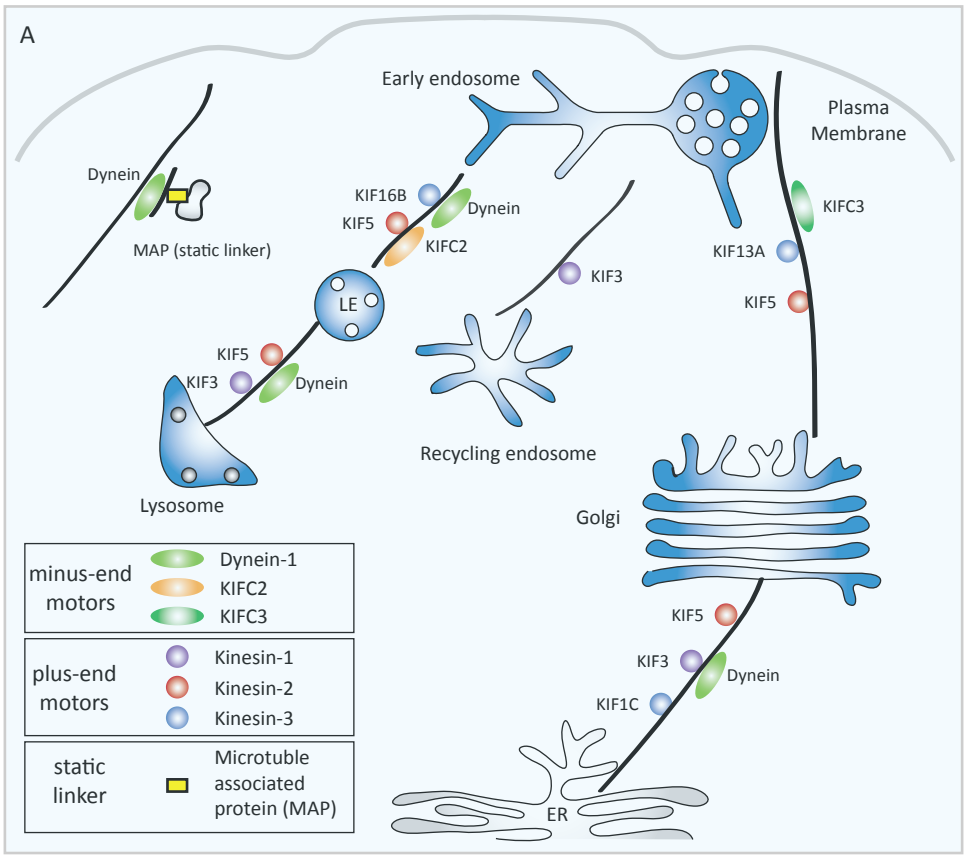
Figure 1: **Membrane and organelle dynamics.** (A) Motors are coupled to almost all cellular membranes to drive membrane traffic between organelles as well as to shape and position organelles within the cytosol. These pathways are discussed in more detail in the text and here we simply illustrate some key example motors that are known to be involved in each step. (B) An example of the dynamic nature of organelles. Stable expression of GFP-tagged sorting nexin 1 (SNX1) in HeLa cells labels early endosomes. Large numbers of puncta are distributed throughout the cytoplasm [see twofold enlargements in (B1), and along with some tubular structures (B2)]. (C) Live cell imaging reveals the dynamics of these structures. The data set has been colour coded according to time (colour scale shown). White indicates structures that do not move during the time sequence); coloured tracks indicate moving objects. Clearly visible within this processed image is the long-range translocation of endosomes in both directions [(C1) note the colour change from red to yellow to green and so on over time) presumably directed by opposing motors. Net movement is consistently centripetal as is clear from the enlargement in (C2) where structures are consistently coloured in the sequence red–yellow–blue–pink, which indicates net movement over time in the same direction (small arrows). Scale bars = 10 μm.

Figure 2: **Motor-protein–membrane coupling affects organelle structure.** (A) Coupling of motors to pleiomorphic organelles can influence their structure and dynamics. Here, dynein (red) and kinesin (green) exert force on distinct domains of the same organelle. This could occur, for example, by virtue of distinct coupling mechanisms. (B) The application of longitudinal force to nascent buds can drive fission. (C) Similarly, force can extend tubules or serve to stabilize tubule formation on such organelles. (D) Opposing motors can generate discrete domains within single membrane-bound structures to assist in cargo segregation to drive sorting during membrane traffic. Please refer to the text for further discussion and examples of these four possible mechanisms, which are, of course, not mutually exclusive.

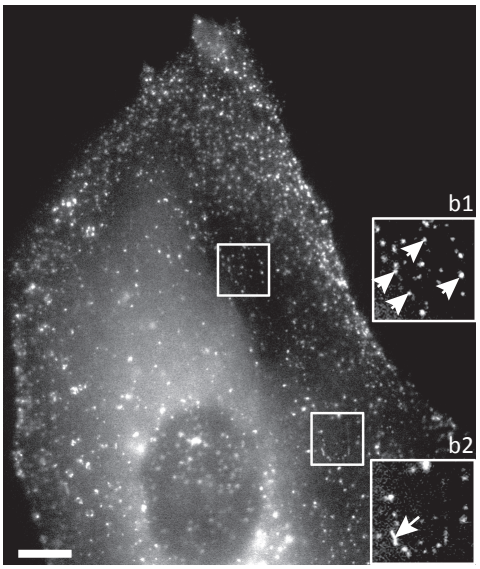
Figure 3: **Nucleation of microtubules at the Golgi.** (A) Nucleation at the *cis* face of the Golgi is driven by AKAP450 in association with GM130 and CLASP2. At the *trans* side (most likely the trans-Golgi network) nucleation is mediated by GCC185, acting in conjunction with CLASP2. (B) AKAP450 and CLASP2 might, in fact, form part of the same mechanistic pathway. Dynamic changes to the Golgi structure as a result of cisternal maturation could result in microtubules that are seeded by AKAP450 at the *cis*-face and then retained through GCC185-dependent mechanisms at the *trans*-face of the Golgi.

Figure for Box 1.





B GFP-SNX1



C GFP-SNX1 90 secs imaging time, 2.3 fps

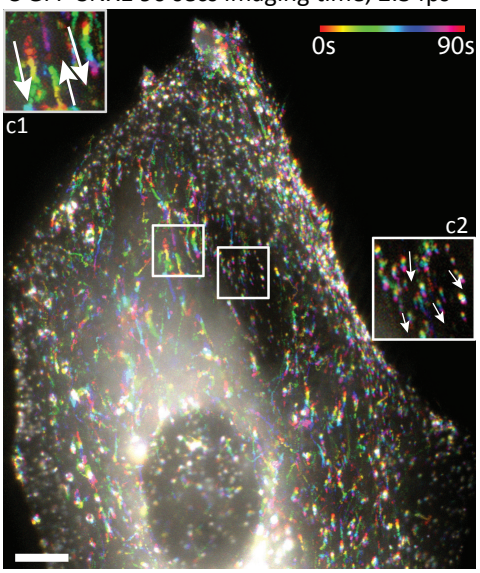


Figure 3

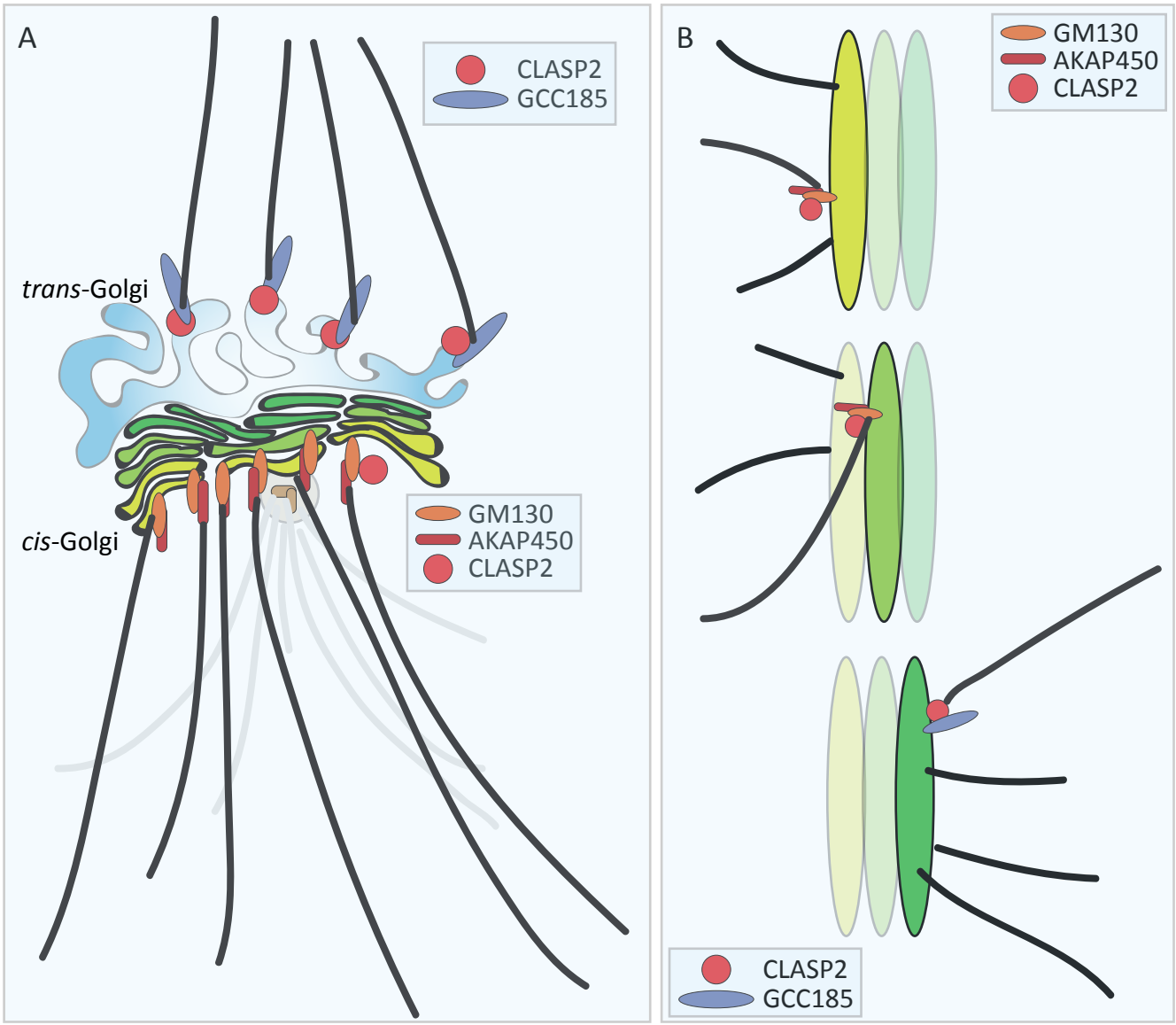
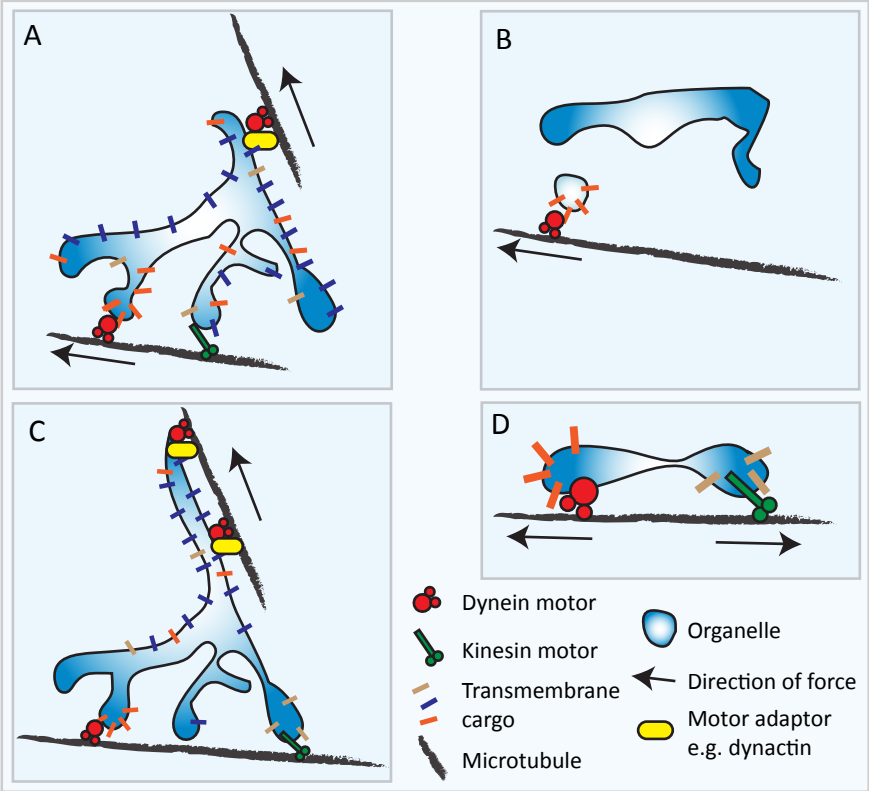

A
CLASP2
GCC185
trans-Golgi
cis-Golgi
GM130
AKAP450
CLASP2
B
GM130
AKAP450
CLASP2
CLASP2
GCC185

Figure 2


A
B
C
D
Dynein motor
Kinesin motor
Transmembrane cargo
Microtubule
Organelle
Direction of force
Motor adaptor e.g. dynactin