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Powering membrane traffic in endocytosis and recycling

Thierry Soldati and Manfred Schliwa‡*

Abstract | Early in evolution, the diversification of membrane-bound compartments that characterize eukaryotic cells was accompanied by the elaboration of molecular machineries that mediate intercompartmental communication and deliver materials to specific destinations. Molecular motors that move on tracks of actin filaments or microtubules mediate the movement of organelles and transport between compartments. The subjects of this review are the motors that power the transport steps along the endocytic and recycling pathways, their modes of attachment to cargo and their regulation.

Endocytosis
A plasma-membrane-associated process in which a eukaryotic cell engulfs extracellular fluid or particles.

Phagocytosis
A form of endocytosis in which a eukaryotic cell engulfs large particles, such as bacteria.

Pinocytosis
A form of endocytosis in which a eukaryotic cell engulfs extracellular fluid and solutes.

Macropinocytosis
A form of pinocytosis, morphologically and mechanistically related to phagocytosis, by which cells form large membrane vesicles.

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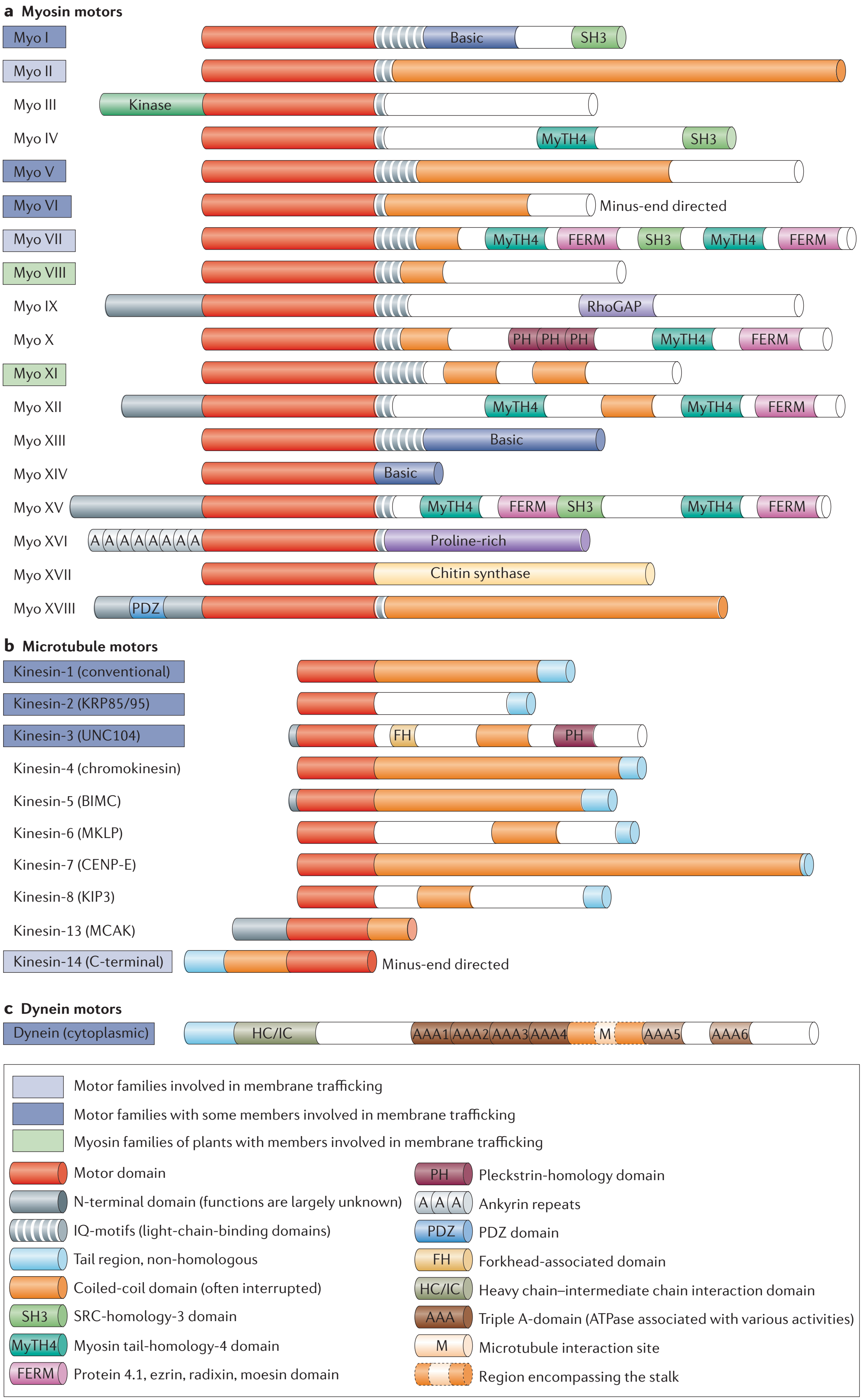
The emergence of eukaryotic cells was accompanied by the development of endomembrane systems that compartmentalize biochemical pathways and biosynthetic processes. An evolutionary trend towards increasing complexity and subcellular specialization necessitated the development of machineries for intercompartmental communication. Molecular assemblies evolved to confer specificity to the interactions of donor and acceptor compartments (budding, sorting, tethering and fusion). Cytoskeletal polymers such as actin filaments and microtubules, which help segregate genetic material and determine cell shape and subcellular architecture, were co-opted as tracks for transport. In animal cells, microtubules support long-range transport, whereas the more flexible actin filaments serve short-range movements both near the cell periphery and in the cell interior. Also, actin filaments can be woven into dense networks of short fibres through the action of crosslinkers and branch-promoting complexes. On the other hand, in many plant cells, bundled actin filaments can form extended tracks for long-distance transport. Owing to the gel-like nature of the cytoplasm, the Brownian movement of organelles is severely restricted and cargoes have to be transported to their destinations at the expense of energy. Furthermore, seemingly random, but motor-dependent, movement is proposed to increase the probability of vesicle collisions and therefore promote fusion events. Movement is powered by three ATP-dependent motors: myosin, kinesin and dynein. Over the course of eukaryotic evolution, these motors have diversified into a large number of families with specific tasks (FIG. 1).

Here, we review the involvement of molecular motors in the movement of endosomes and in vesicular trafficking along the endocytic and recycling pathways. These pathways are summarized in FIG. 2. We do not, however, attempt to consider details of the secretory pathway nor trafficking related to other organelles such as mitochondria, chloroplasts or peroxisomes. We follow the journey of endocytic compartments on their inbound route from the plasma membrane to their centrally located destinations and then accompany recycling compartments back to the surface. Most of the data referred to have been acquired from animal systems, except where mentioned otherwise. The basic properties of molecular motors, such as how they generate movement and tension, have been discussed in a compilation of reviews elsewhere[1], although some of the salient features are summarized in BOX 1. For a discussion of the additional mechanisms by which motors produce movement, such as harnessing the power of actin polymerization, readers are referred to recent reviews[2,3]. The diversity of the motors that are engaged in these processes is reflected in the complexity of attachment modes to their diverse cargoes, which are summarized in BOX 2. More complete outlines of cargo-binding mechanisms can be found in recent reviews[4–6]. Here, we emphasize the interplay between endocytic and recycling traffic, and the requirement for molecular motors in these dynamic processes.

Inbound traffic

Endocytosis probably evolved as a process to take up nutrients from the environment and to avoid the need to secrete hydrolytic enzymes for the purpose of extracellular digestion. Endocytosis can be divided into phagocytosis, which is the uptake of particles, and pinocytosis, which is the uptake of fluid. Depending on the type of particle and the receptors that are required, several morphological and functional modes of phagocytic uptake have been described. Furthermore, there are four basic mechanisms for pinocytosis: macropinocytosis,


a Myosin motors
Myo I
Basic
SH3
Myo II
Myo III
Kinase
Myo IV
MyTH4
SH3
Myo V
Myo VI
Minus-end directed
Myo VII
MyTH4
FERM
SH3
MyTH4
FERM
Myo VIII
Myo IX
RhoGAP
Myo X
PH
PH
PH
MyTH4
FERM
Myo XI
Myo XII
MyTH4
MyTH4
FERM
Myo XIII
Basic
Myo XIV
Basic
Myo XV
MyTH4
FERM
SH3
MyTH4
FERM
Myo XVI
A A A A A A A A
Proline-rich
Myo XVII
Chitin synthase
Myo XVIII
PDZ
b Microtubule motors
Kinesin-1 (conventional)
Kinesin-2 (KRP85/95)
Kinesin-3 (UNC104)
FH
PH
Kinesin-4 (chromokinesin)
Kinesin-5 (BIMC)
Kinesin-6 (MKLP)
Kinesin-7 (CENP-E)
Kinesin-8 (KIP3)
Kinesin-13 (MCAK)
Kinesin-14 (C-terminal)
Minus-end directed
c Dynein motors
Dynein (cytoplasmic)
HC/IC
AAA1
AAA2
AAA3
AAA4
M
AAA5
AAA6
Motor families involved in membrane trafficking
Motor families with some members involved in membrane trafficking
Myosin families of plants with members involved in membrane trafficking
Motor domain
N-terminal domain (functions are largely unknown)
IQ-motifs (light-chain-binding domains)
Tail region, non-homologous
Coiled-coil domain (often interrupted)
SH3 SRC-homology-3 domain
MyTH4 Myosin tail-homology-4 domain
FERM Protein 4.1, ezrin, radixin, moesin domain
PH Pleckstrin-homology domain
A A A Ankyrin repeats
PDZ PDZ domain
FH Forkhead-associated domain
HC/IC Heavy chain–intermediate chain interaction domain
AAA Triple A-domain (ATPase associated with various activities)
M Microtubule interaction site
Region encompassing the stalk

Clathrin-mediated endocytosis
A form of pinocytosis, often also referred to as receptor-mediated endocytosis, in which the invagination of the endocytic vesicle is driven by the clathrin coat.

Caveolae-mediated endocytosis
A form of pinocytosis that is driven by a coat made of the protein caveolin.

Clathrin- and caveolae-independent endocytosis
A form (or forms) of uptake that is revealed when the two other endocytosis pathways are blocked.

Lamellipodia
Sheet-like plasma-membrane protrusions that are formed by actin polymerization at the leading edge of motile cells.

Phagocytic cup
A bowl-shaped lamellipodia-like protrusion that forms around particles during phagocytic uptake.

Processivity
The capability of a single motor to move for long distances without dissociating from the track.

clathrin-mediated endocytosis, caveolae-mediated endocytosis and at least one less well-defined clathrin- and caveolae-independent endocytosis pathway. Recent reviews describe these portals of entry into the cell in detail[7,8].

Phagocytosis. Phagocytosis involves the active protrusion of lamellipodia around an object that is to be engulfed, and several myosin classes are involved in this process. Myosin Ic[9] and myosin X[10] seem to be key players in the extension of phagocytic cup pseudopods, whereas myosin II promotes closure of the cup[11] (FIG. 2). Microtubule motors might contribute to phagosome formation by delivering endomembranes to the nascent phagocytic cup, although the motors that could be involved have not yet been identified. In the amoeba *Dictyostelium discoideum*, various myosin-I motors have been localized to the phagocytic cup, and their overexpression or deletion affects the uptake of particles[12] (reviewed in REF. 13). It has recently been proposed, for both amoeba and yeast, that myosin-I motors function not in transport *per se*, but rather in the regulation and organization of the actin nucleation–polymerization machinery[2,3]. Whether this might also be true for animal cells has not as yet been studied. Myosin VII has been implicated in phagocytic uptake both in *D. discoideum* and mammalian cells (reviewed in REF. 14). Again, it does not seem to be involved in phagosome transport *per se*, but rather is proposed to have a conserved role in the formation of adhesion complexes[15,16] that are essential for particle (and substratum) binding. Myosin VIIb from *Drosophila melanogaster* and *Mus musculus* is bound to filamentous (F)-actin for about 80% of its ATPase cycle. The processivity of myosin VIIb (BOX 1) is similar to that of myosin V and VI, which is compatible with it having a role in long-range transport[17]. However, slow ADP release and a turnover rate that is much slower than that of myosin VI actually favours myosin VIIb having a role in the generation and maintenance of tension[18]. Last, the finding that myosin VIIa in inner ear sensory cells interacts directly with the pleckstrin-homology domain retinal protein-1 (PHR1), an integral membrane protein that also binds to myosin Ic, supports a role for both of these myosins in anchoring the plasma membrane to the underlying actin cytoskeleton[19].

Following internalization, myosin V probably controls short-range phagosome movement and delivers phagosomes to the microtubule system for transport mostly (but not exclusively) in the minus-end direction[20] (discussed below).

Macropinocytosis. Macropinocytosis is the bulk uptake of extracellular medium through large, goblet-shaped invaginations; a process that morphologically and mechanistically resembles phagocytosis. In the freshwater amoeba *Acanthamoeba*, the three myosin I isoforms associate with macropinocytic cups in a way that is similar to their association with phagocytic cups[21]. A similar situation has been observed in *D. discoideum*[22] (reviewed in REF. 13). Class-I myosins are known to associate with membranes directly (BOX 2) by binding to acidic phospholipids in a Ca^{2+}-dependent manner, and they probably remain associated through numerous rounds of ATP hydrolysis[23]. Myosin dissociation from phagosomes and macropinosomes occurs rapidly after pinching off from the plasma membrane. Whether the myosins participate in organelle transport or instead are required for functions linked to actin nucleation–polymerization remains unclear[1]. In *D. discoideum*, microtubules facilitate the fusion of macropinosomes with other endosomes[24], and subsequent transport is dynein-dependent. In mammalian cells, recent evidence implicates both dynein light chain-1 (DLC1) and p21-activated kinase-1 in the initiation of pinosome formation and trafficking, indicating an early involvement of microtubule motors in pinosome formation[25].

Clathrin-mediated endocytosis. The involvement of the actin cytoskeleton in clathrin-mediated endocytosis in animal cells and lower eukaryotes has long been a matter of debate. Recent work in *Saccharomyces cerevisiae* shows that the energy of actin nucleation–polymerization is harnessed for this mode of uptake[2]. Although the machinery involved is highly conserved in most eukaryotes, the contribution of the actin system in animal cells is less clear. In *S. cerevisiae*, the involvement of clathrin is proposed to be limited to the initiation of endocytic-site assembly rather than membrane invagination or vesicle formation[26]. On the other hand, actin and class-I myosins are essential for internalization (FIG. 2). Class-I myosins are important for the scission step[27], whereas the actual movement away from the cell periphery is powered by actin polymerization[26]. By contrast, the role of myosin I in *Schizosaccharomyces pombe* seems to be restricted to the organization of sterol-rich plasma-membrane domains in a manner that is independent of endocytosis[28]. The polybasic tail-homology domain-1 (TH1) of class-I myosins binds directly with high affinity to acidic phospholipids[23]. Most interactions of myosins with their cargoes occur through binding sites in the tail region (FIG. 1; BOX 2). One striking exception is the yeast UCS-domain protein She4, which binds to the motor domains of a class-V myosin (specifically, Myo4) as well

◀Figure 1 | **The structural diversity of molecular motors.** Mammals have genes for over 100 motors, and any given cell might express more than 50 of them. On the basis of various criteria, both the myosin (Myo) and the kinesin motors can be subdivided into families of distinct sequence organization and cellular function. **a** | From the most recent census, the myosin superfamily comprises 24 classes[136], of which 18 are shown here because they have members that have, to a certain degree, been characterized (the recently proposed classes 19–24 are mainly derived from *in silico* work). **b** | A new kinesin nomenclature[152] now lists 14 kinesin families, 10 of which are shown (old nomenclature in parenthesis). **c** | Dyneins can be subdivided into at least 15 subfamilies, of which 14 are involved in the movement of cilia and flagella, and 1 is involved in cytoplasmic transport[153] (shown). So far, only one class each of the myosins and kinesins have family members that move towards the minus end of actin filaments or microtubules, respectively. Myosin and kinesin families harbour some common structural domains and all contain the signature motor domain (see key). In addition, some domains are unique to particular family members. For example, a kinase domain is found in myosin III, a proline-rich region is found in myosin XVI, a RhoGAP domain is unique to myosin IX and a chitin-synthase domain is found only in myosin XVII. Three myosin families (I, XIII and XIV) have regions that are rich in basic residues and might be involved in binding to membrane lipids of cargo vesicles.

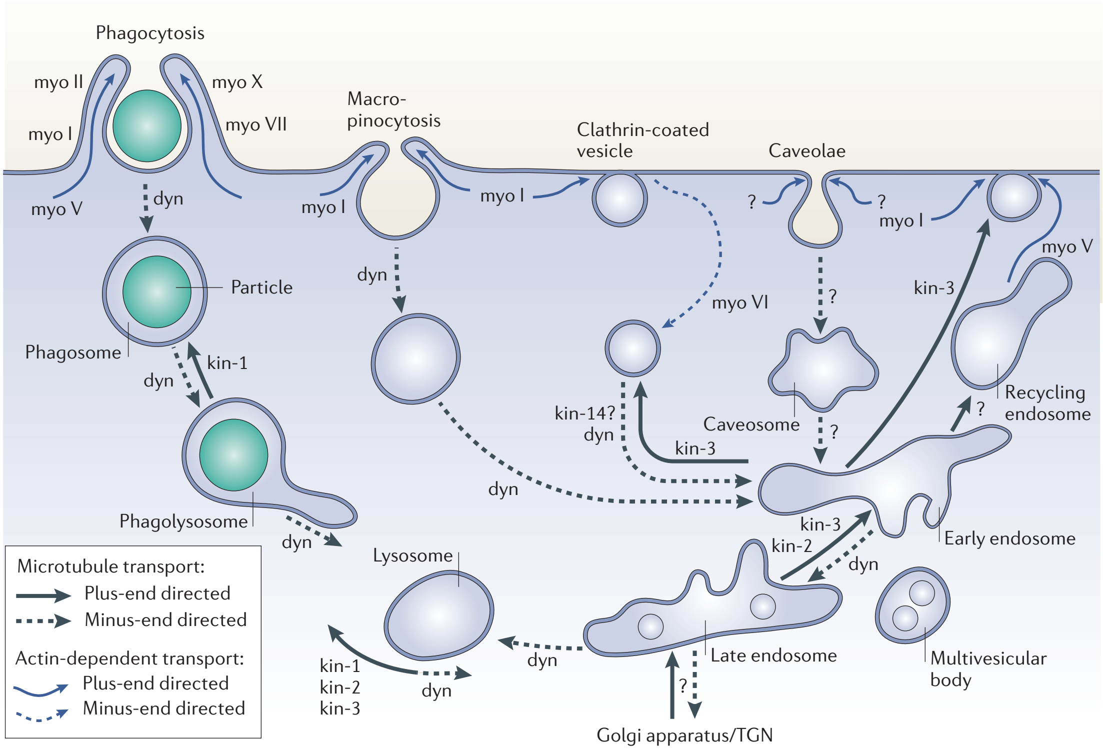


Figure 2 | **Overview of the motors that are involved in endocytic and recycling traffic.** The figure is compiled from studies that used a wide range of organisms, from amoebae to mammals. Endocytic cargoes are transported around cells along actin filaments or microtubules. The different classes of myosin (myo), kinesin (kin) or dynein (dyn) that have been implicated at each step are shown. In the three pinocytic pathways, macropinocytosis, the clathrin-dependent pathway and the caveolae pathway, internalized vesicles are transported to the early sorting station (early endosome). In the caveolar pathway, a compartment before the early endosome compartment, the caveosome, has been identified. Cargoes travel from early to late endosomes via endosomal carrier vesicles, also called multivesicular bodies, because they usually acquire internal membrane profiles as a result of the inward invagination of the delimiting membrane. The cargoes that are destined for degradation then continue their journey to lysosomes, whereas others are recycled either to the plasma membrane (directly or through recycling endosomes) or to the Golgi apparatus (specifically to the *trans*-Golgi network (TGN)). Subsequently, these cargoes, together with newly synthesized proteins, can exit the TGN and traffic either to the plasma membrane or to late endosomes, and to a minor extent to early endosomes (not shown). Solid particles are taken up by phagocytosis into phagosomes, which are similar to the early endosome compartments of the pinocytic pathway. Phagosomes progressively mature into phagolysosomes, which are similar to late endosomes, and then lysosomes. Motor-dependent, bidirectional movement of lysosomes is also shown.

as class-I myosins (specifically, Myo3 and Myo5)[29]. She4 seems to be required for both the proper localization of class-I myosins at endocytic sites and for class-V myosin function.

Contrary to the more indirect role of class-I myosins, myosin VI is directly involved in the formation of clathrin-coated vesicles (CCVs) at the apical intermicrovillar region of the mammalian brush border[30] (reviewed in REF. 31) and in their movement along cortical actin filaments after clathrin uncoating[32] (FIG. 2). Myosin VI is the only myosin that has been shown so far to move towards the minus end of actin filaments (FIG. 1). Therefore, it might be essential for the transport of nascent endocytic vesicles from the actin-rich cell periphery to early endosomes[32], by delivering them to the microtubule-transport system. It is worth noting that class-VI myosins have been found only in animal cells, and they therefore seem to be dispensable for clathrin-mediated endocytosis in lower eukaryotes. Although myosin VI is a monomer in solution, it nevertheless can move processively along actin filaments with surprisingly long strokes[33]. Dimerization was found to be regulated by the clustering of myosin VI on the surface of its vesicular cargo[34], reminiscent of the mechanism that was proposed for the kinesin UNC104 (REF. 35). Recently, the processivity of myosin VI was shown to be markedly enhanced when it was attached to an artificial bead cargo, and this was further increased by high viscosity[36]. This led to the proposal that the large size of a cargo can function as a 'diffusional anchor', enhancing the rebinding of myosin heads following their detachment from actin filaments.

Adaptor proteins assist the motors in these tasks. For example, disabled-2 (DAB2), a clathrin-binding protein, recruits myosin VI to nascent CCVs[31], whereas gp75-interacting protein (GIPC) binds to the C terminus of glucose transporter-1 (GLUT1)[37] and probably recruits myosin VI to uncoated vesicles[38]. DAB2 might link, directly or indirectly, the myosin VI–clathrin complex

Acanthamoeba
A genus of small, highly motile soil amoebae that are frequently used for studies of actin-binding proteins and cell locomotion. Some pathogenic forms are known.

Minus end (plus end) direction
Both actin filaments and microtubules demonstrate polarity, and are assembled from monomers that are added at a high rate at one end (the plus end) and at a much slower rate at the other (the minus end). Motors can transport cargo in either direction, processes that are referred to as plus-end-directed and minus-end-directed transport.

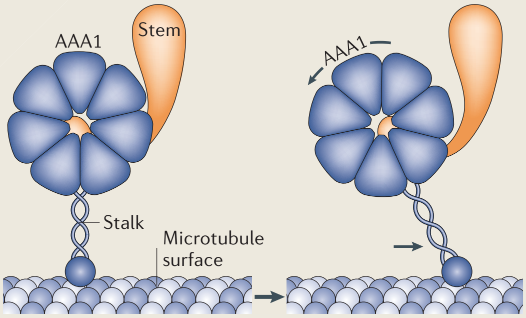
Box 1 | **Properties of molecular motors**

The three classes of motor — myosin, kinesin and dynein — share several features[128]. First, their motor domains use the energy derived from ATP hydrolysis to undergo characteristic conformational changes to generate a 'step' along the track. These changes are similar in myosins and kinesins, but different in dyneins. The step size ranges from a few nanometres for myosin II, to 8 nm for kinesins and dyneins, to 36 nm for myosin V and VI. Second, non-motor domains are important for motor function, including coiled-coil segments for dimerization and regulatory or cargo-binding domains. Last, motors can generate a force of several picoNewtons that is sufficient to move even large objects through a crowded cytoplasm.

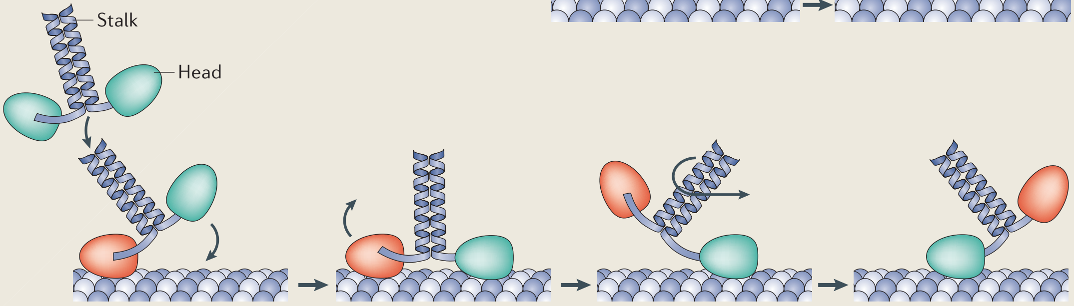
The crystal structures of myosin and kinesin have uncovered an unexpected structural similarity, indicating that they have a common evolutionary origin[129]. By contrast, dynein is an AAA+ (ATPases associated with various cellular activities) protein. Six AAA+ modules and a seventh non-AAA+ domain are arranged in a ring and form the seven-lobed dynein head[130]. The first four AAA+ domains have an intact P-loop motif that signifies a nucleotide-binding site. The first AAA+ domain (AAA1) is the primary site of ATP hydrolysis, although all four functional sites seem to cooperate[131]. The microtubule-binding site is located at the end of a unique ~10-nm stalk that extends from the side that is opposite the first AAA+ unit. Force generation is proposed to involve a rotation of the entire head[132] (panel **a**).

The task of moving cargo around the cell is intimately linked to the capability of a single motor to move for long distances without dissociating from the track — a property that is termed processivity. Processive motors are dimers that have, at any given time, one head firmly bound to the track while the other is stepping towards the next binding site[133]. Examples of processive motors are kinesin-1 (panel **b**), myosin V, myosin VI and cytoplasmic dynein.

a **Rotation of the dynein head**



b **Stepping of kinesin-1**



to transmembrane proteins such as the CFTR (cystic fibrosis transmembrane conductance regulator) channel[39] and possibly also to members of the low-density lipoprotein (LDL)-receptor family[40]. In the brain, myosin VI exists in a complex with aminomethyl propionic acid (AMPA) receptors, a subtype of glutamate receptor. Therefore, the motor seems to function in the clathrin-mediated endocytosis of this receptor through binding to its GluR1 subunit[31]. The loss of myosin VI might contribute to the Snell's waltzer phenotype by leading to alterations in synaptic structure[41].

The vesicle-forming machinery at the plasma membrane shows striking similarities to the machinery of the Golgi apparatus[42]. These two machineries are at opposite ends of the microtubule-transport system. The actin cytoskeleton is tightly associated with both organelles, as are myosin II and class-I myosins. At both sites, adaptor complexes and clathrin are used to shape the vesicles, and myosin VI seems to function in vesicle formation and delivery to the microtubule system. A strong candidate for a binding partner for myosin VI on plasma-membrane-derived vesicles as well as at the Golgi apparatus is optineurin[43], a conserved protein that was first identified as a binding partner of an adenoviral protein. In fibroblasts, the absence of myosin VI was shown to be responsible for the significant reduction in the size of the Golgi and in the level of secretion[44], as well as the impairment in the process of exocytosis, confirming its importance in Golgi function and beyond[43].

Caveolar endocytosis. Caveolae are flask-shaped invaginations of the plasma membrane that are lined by caveolin. It has long been debated whether they can bud from the surface and transport cargoes to endosomes, but now it seems that this process is cell-type dependent and can be stimulated (reviewed in REFS 8,45). In mammalian cells, local actin polymerization accompanies the formation of caveolae and is thought to assist in their internalization, probably powered by an actin tail[46], but no myosin has yet been shown to be involved. Caveolae are the port of entry for some glycosylphosphatidylinositol-anchored proteins and viruses such as simian virus-40. Activation of caveolae by simian virus-40 enhances the fraction of newly formed caveolar vesicles and caveosomes[47].

Snell's waltzer phenotype
A mouse mutation in myosin VI that arose spontaneously at the Jackson laboratory in the 1960s. It affects inner ear structure and leads to deafness and vestibular dysfunction, causing the mice to circle.

Caveosomes
Endocytic vesicles enriched for the protein caveolin-1 formed from caveolae, flask-shaped pits in the membrane that resemble a cave.

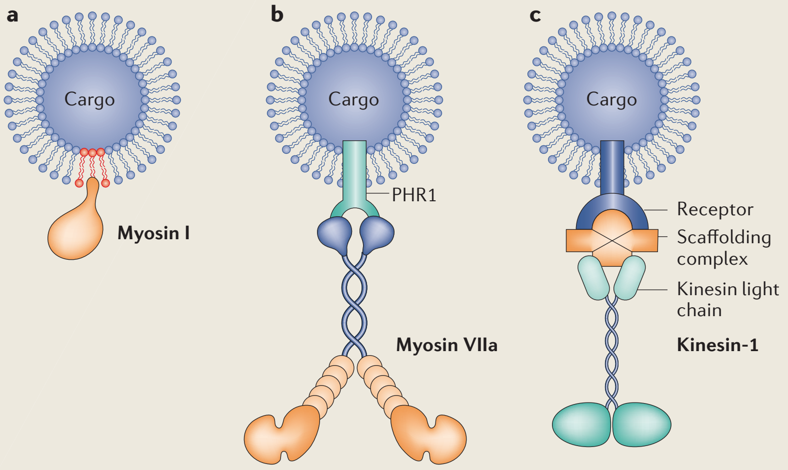
Box 2 | Modes of motor–cargo association

Only a subset of myosin, kinesin and dynein motors have so far been shown to power vesicular trafficking (FIG. 1). Cargo association involves non-motor domains and, in many cases, accessory proteins. Three types of cargo-association mechanism can be distinguished.

Association with membrane lipids. Essentially all of the class-I myosins have a polybasic domain that has an affinity for membranes[23] (panel **a**). Some members of the kinesin-3 family, such as UNC104, bind to phospholipids directly through their pleckstrin-homology (PH) domain and can cluster in lipid rafts, facilitating dimerization[35]. PH domains are also present in myosin X, but it is not known whether this motor is involved in vesicle movement. *Dictyostelium discoideum* MyoM contains a Rac guanine-nucleotide exchange factor domain that has a PH subdomain[134] and associates with macropinocytic structures[135]. Last, genome mining revealed a human myosin that contains a FYVE domain that is thought to interact with endosomal phosphatidylinositol-3-phosphate[136].

Binding to integral membrane proteins. Examples include kinesin-1, which has been proposed to interact directly with amyloid precursor protein[137]; however, data remain controversial[138]. Myosin Va interacts with syntaxin-1A in a Ca^{2+}-dependent manner to regulate exocytosis[106]. In inner ear sensory cells, myosins Ia and VIIa interact with the integral membrane protein pleckstrin-homology domain retinal protein-1 (PHR1)[19] (panel **b**).

Linkage through scaffolding complexes. This is thought to be the most widespread form of membrane–motor interaction. Kinesin-1, -2 and -3 (panel **c**) as well as myosin V and VI can bind to multisubunit adaptors that link them to membranes. A bulky activator complex, dynactin, is likely to be involved in regulating dynein targeting and recruitment to vesicular cargo, although the molecular details of its interactions have yet to be worked out[66]. Dynactin can bind to phospholipids and membrane-associated spectrin, providing a possible means for interaction with multiple membrane cargoes[139]. However, spectrin is not a crucial component of cargo binding in *Caenorhabditis elegans* neurons[140]. RAB proteins might be involved in cargo binding as well. For example, at the Golgi apparatus, RAB6, its interactor Bicaudal-D and Golgi-specific β-III spectrin interact with distinct dynactin subunits and/or directly with dynein[141], whereas at phagosomes, RAB7 and its interactor RAB7-interacting lysosomal protein (RILP) recruit the dynein–dynactin complex[68].



Activation also results in the increased long-range transport of caveolar vesicles along microtubules, which indicates that an as-yet-unidentified microtubule motor is active.

Subsequent transport steps. After uptake, two conceptually different models have been proposed to account for the modification of endosome (phagosome or pinosome) composition and function. In one, organelles are compositionally stable stations along a set pathway and components are transported between them by shuttling vesicular carriers. Alternatively, the organelle itself gradually changes its composition and acquires a different identity as it follows a 'maturation programme'. In phagocytosis, as large objects cannot be squeezed into standard-sized vesicles, maturation seems to be plausible and is widely accepted. By contrast, the pinocytic pathway is frequently assumed to use a shuttling system, although the recently proposed RAB-conversion mechanism is reminiscent of a maturation process[48].

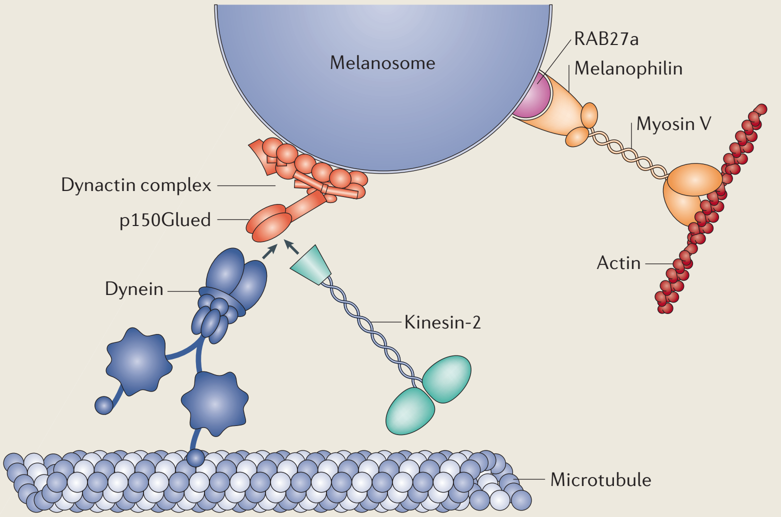
Newly formed phagosomes[49] and macropinosomes[50,51], the largest forms of endosomes, are propelled by an actin comet tail towards the cell centre, but myosin has not been directly implicated in this movement. Actin and myosins are associated with endosomes and lysosomes, but their role in transport between these organelles is probably not direct[52]. They seem to cooperate with microtubule motors to steer the long-range movement of lysosomes, because the disruption of actin or the expression of a dominant-negative form of myosin Ib increased lysosome mobility but impaired long-range directionality[52]. Similarly, overexpression of GFP-myosin Ib induced perinuclear clustering of multivesicular body endosomes[53].

In contrast to their formation, the subsequent transport of endosomes calls for their linkage to microtubules, which requires the cooperation of microtubule and actin-based motors. Based on the observation that the same vesicle can actually switch between microtubules and actin filaments in the squid axoplasm[54], it is likely that myosins drive short-range movements through actin-rich regions either preceding (as in endocytosis) or following (as in recycling and exocytosis) long-range transport along microtubules. A cell system that serves as a model for the coordination of different superfamily motors is the melanophore (BOX 3). Although melanosomes are not endocytic vesicles *per se*, they belong to the emerging category of exocytic lysosome-like organelles, so their mode of association with microtubules and actin-based motors might nevertheless be of relevance. In the endocytic route, endosomes are delivered to the microtubule system. How exactly, in molecular terms, can the process of delivery to microtubules be described? Somehow, endosomes must be linked to minus-end-directed microtubule motors. Recent studies show that a set of evolutionarily conserved proteins specifically accumulate at microtubule plus ends (reviewed in REF. 55). These microtubule-plus-end-tracking proteins (+TIPs) regulate microtubule dynamics and interactions with the cell cortex. Intriguingly, +TIP complexes include, among others components, dynein and the p150Glued subunit of the dynein activator complex, dynactin. This has led to the attractive hypothesis that dynactin that is associated with +TIPs can load membrane cargo onto microtubules for subsequent transport by dynein[56]. Indeed, another +TIP component, cytoplasmic linker protein (CLIP), was originally identified as a protein that links endocytic vesicles to microtubules *in vitro*[57]; but, the function of CLIP *in vivo* is unclear. Downregulation of CLIP170, which reduces dynactin at microtubule tips, does not

Box 3 | **Coordinating several motors: the melanosome paradigm**

The capability of organelles to switch from forward to reverse movement and back again is linked to the presence and coordination of different motors on the same organelle. The best-studied model system, and one that functions as a paradigm for other transport phenomena, is the melanosome of vertebrate cells (reviewed in REF. 142). In amphibians, melanin-containing vesicles known as melanosomes can be either dispersed throughout the cell or aggregated in the cell centre, thereby producing changes in coloration. Dispersion and aggregation are driven by kinesin-2 and cytoplasmic dynein, respectively (see figure). Novel precision measurements indicate that 1 or 2 copies of kinesin-2 and 1–3 dyneins can move a melanosome[84]. Kinesin-2 and dynein compete for the same binding site on p150Glued of dynactin[143] (arrows in the figure) and also interact with the regulator of melanophore activity, protein kinase A (not shown)[144]. In addition, the actin cytoskeleton might assist kinesin-2 in transporting or anchoring melanosomes at the cell periphery by being delivered to myosin V motors. During aggregation, the release of myosin V helps the dynein-mediated movement to 'win' over the kinesin-2-mediated movement[145]. In mammalian melanocytes, myosin V is recruited to melanosomes by RAB27a through melanophilin, a RAB-binding protein[146], in preparation for melanosome transfer to keratinocytes. In this cell type, myosin V and melanophilin track the microtubule plus end through an interaction with microtubule-plus-end-binding protein-1 (EB1)[147]. As melanosomes are lysosome-related organelles, the prominent bidirectional movements of lysosomes might be driven by a similar motor complex that involves kinesin-2 (REF. 79). Generally speaking, the melanosome paradigm offers an explanation for the frequent finding of the 'wrong' motor on organelles that are travelling in a particular direction[80].

An analogous complex of RAB27a, a melanophilin-related protein (SLAC2C, also known as MYRIP), and myosin VIIa (another myosin motor) has also been identified on melanosomes in retinal pigmented epithelial cells in mammals[148]. The same complex is present on secretory granules in β-cells (see REF. 149). The association of myosin V[106,127], RAB27a[150], or both[151] with other types of exocytic granule hints at a myosin–scaffolding protein–RAB27 machinery that drives the late steps of exocytosis in many instances.



RAB
One family of the large superfamily of small GTP-binding proteins involved in a myriad of cellular functions. RAB proteins are best known for their role in the timing of vesicle fusion.

affect organelle distribution and organism survival, arguing against a role for +TIP-associated dynactin in vesicle trafficking[58]. In *D. melanogaster*, the homologue of CLIP170, DCLIP190, has been reported to bind to myosin VI[59], which might indicate a direct link between the actin-based motor system and the ends of microtubules. The precise relationships between +TIP-associated motors, vesicle loading and microtubule dynamics require further examination[60].

Unquestionably, endocytic vesicles initially travel towards the microtubule minus end. These movements are potentially driven by two motors: cytoplasmic dynein and/or a C-terminal minus-end-directed kinesin, such as kinesin-14. *In vitro* studies of endocytic vesicles from rat liver indicated an involvement of the minus-end-directed kinesin, KIFC2 (REF. 61). However, KIFC2-knockout mice are viable and reproduce normally, arguing against a crucial role of KIFC2 for cell viability[62]. Moreover, KIFC2 is involved, if at all, in the movement and fission of early endocytic vesicles, whereas late vesicles are associated with the dynein–dynactin complex as a minus-end-directed motor[63]. The dynein–dynactin complex therefore seems to be the chief motor for minus-end-directed endocytic traffic[64–67] (FIG. 2).

An important player in the linkage of the motor complex to late endocytic compartments is the small GTPase RAB7. Its two effectors, RAB7-interacting lysosomal protein (RILP) and oxysterol-binding protein (OSBP)-related protein-1L (ORP1L), recruit the dynein–dynactin complex to these compartments, promoting dynein-mediated tubulation[68] and transport[69–71]. A potential site in RILP that is implicated in motor recruitment has been identified[72], although it is still unclear whether RILP interacts directly with dynein. Another potentially important contribution to dynein function might be made by a different, unexpected player, the protein Zeste white-10 (ZW10), a mitotic checkpoint protein and an anchor for dynein at the kinetochore[73]. In interphase cells, RNA interference (RNAi)-mediated knockdown of ZW10 not only causes Golgi scattering, but also causes dispersal of endosomes and lysosomes and a reduction in minus-end-directed movements. Therefore, ZW10 might have a general role in dynein regulation, targeting and cargo binding.

Plus-end-directed motors also have a role in vesicle movement, because in many cell systems the movement of vesicles in the endosomal pathway is bidirectional (FIG. 2). Bidirectionality is most pronounced at the late endosome step, but can also occur shortly after endocytosis. In mammalian cells, the movement of early endosomes can be counteracted by the plus-end-directed kinesin-3 motor KIF16B, which transports early endosomes in a RAB5-dependent manner[74]. *In vivo*, overexpression of KIF16B causes the relocation of early endosomes to the cell periphery and inhibits their transport to the degradative pathway. The main function of the motor might be to regulate the balance between receptor recycling and degradation. A fungal-specific kinesin-3 motor drives early endosome movement in yeast-like hyphae of the filamentous fungus *Ustilago*[75]. In the hyphae, endosomes are moved to the hyphal apex by kinesin-3, where they are loaded onto dynein motors for reverse movement[76]. Uploading of endosomes onto dynein is dependent on dynactin and LIS1 (a dynein activator that is defective in patients with the brain disorder lissencephaly[77,78]), which both accumulate at microtubule plus ends. The functional significance of bidirectional endosome movement needs to be clarified, but it might be either to keep these organelles dispersed in the cytoplasm[79], to enhance encounters between different compartments for subsequent fusion[68], or to support fission[61].

Opposite directionality motors are not in a tug of war, but rather seem to be differentially activated and inactivated (for a review, see REF. 80), although this has not been shown for all forms of bidirectional motility. A curious phenomenon that is frequently observed in various cell systems is a block of movement in both directions when one of the motors (for example, cytoplasmic dynein) is downregulated[81,82]. This could be explained by the close association of both classes of motor on their cargoes, which conceivably can occur either by direct interaction[83] or coordination on the membrane[76,84] (see below and BOX 3). A potentially new twist to the story of bidirectional transport is the recent finding that purified dynein–dynactin complexes can move bidirectionally on microtubules *in vitro*[85]. This does not agree with the prevailing dogma that one type of native motor always moves in one direction only. This finding certainly needs to be confirmed in an *in vivo* situation in which dynein transports a membrane-bound cargo. A bidirectional function of dynein would offer an explanation for the aforementioned inhibition of bidirectional transport on the downregulation of dynein. However, studies of lysosome trafficking in a dynein-binding-defective mutant of mammalian cells do not support this suggestion; although a significant reduction in minus-end-directed movements was detected, plus-end-directed motility was unaltered[86].

In summary, the initial steps of endosome transport entail a choreography of myosin motors, microtubule attachment and dynein-mediated movement. This is followed by bidirectional transport along microtubules, which involves dynein and kinesins. It is unclear, however, whether the motors directly interact with one another, but the small G-protein RAB7 is a likely candidate for a regulator that switches the movement of cargoes between minus-end directed and plus-end directed[87].

Outbound traffic

Recycling pathways of endocytosed materials and components of the cell surface might involve several sorting events that take place at different steps of the pathway[88]. The major sorting and recycling pathway begins at the early (also known as the sorting) endosome, from where cell-surface components can be recycled back to the plasma membrane in a fast recycling step. Alternatively, cargo can be delivered to a longer-lasting membrane system known as the endocytic recycling compartment from where materials are returned to the cell surface in a slow recycling step. All of these compartments are dynamic and molecules constantly flow through them so that typical 'resident' proteins are difficult to define. From early endosomes, materials can be delivered to a separate membrane compartment called late endosomes. Late endosomes can be quite heterogeneous, although the functional basis of this heterogeneity is not fully understood. From late endosomes, materials can be further delivered to lysosomes or the *trans*-Golgi network (TGN). Communication between these compartments occurs through fission and fusion events with intercalated transport steps.

Although recycling is commonly thought to begin at the early endosome, an unusual pre-early endosome sorting and recycling step has recently been proposed. This step is initiated at clathrin-coated vesicles and requires microtubule-dependent movement[89], but the motors that are involved are presently unknown.

Long-range delivery. Whereas dynein is responsible for maintaining endosome localization near the cell centre, plus-end-directed kinesins probably transport vesicles from the perinuclear endocytic compartment to the cell surface (FIG. 2). Kinesin-1 is proposed to power the export of vesicles from the endocytic recycling compartment to the plasma membrane[90]. On the other hand, RAB5-dependent transport of early endosomes to the cell periphery is mediated by KIF16B, a member of the kinesin-3 family[74]. The overexpression of this motor provokes a peripheral localization of early endosomes, whereas its ablation causes perinuclear clustering. KIF16B is therefore responsible for early-endosome localization and modulates receptor recycling and degradation.

Plus-end-directed transport of late endosomes (but not early or recycling endosomes) is powered by a member of the kinesin-2 family of heterotrimeric motors[79]. The same study indicated that this motor is also involved in the transport of lysosomes[79], whereas previous studies implicated a splice variant of the kinesin-3 motor KIF1B[91] or members of the kinesin-1 family of conventional kinesins in lysosomal movements (reviewed in REF. 92). In mammalian cells, a rigor mutant of kinesin-1 blocks plus-end-directed lysosome movement[93], and targeted disruption of the gene that encodes kinesin-1 impairs lysosome dispersion[94]. Last, an intriguing finding is that KIF2β, a splice variant of KIF2 that is found in non-neuronal cells and a member of the kinesin-13 family, supposedly drives plus-end-directed transport of lysosomes[95]. This is odd because other members of the kinesin-13 family are non-motile and possess microtubule-depolymerizing activity[96]. Whether all three classes of kinesins are active on a given organelle, whether their involvement is cell-type specific and whether involvement depends on the state of activity of the cell or organelle in question still remain unclear.

The recycling of GLUT4 is another interesting case. Insulin regulates glucose uptake by promoting the transfer of sequestered GLUT4 from perinuclear storage vesicles (that are probably part of a regulated recycling-endosome system) to the plasma membrane. This process is dependent on microtubule-based transport, although which motor is involved is controversial. Dominant-negative mutants of kinesin-1 light chain blocked plus-end-directed transport, which indicates an involvement of kinesin-1 (REF. 97). In another study, transport is proposed to require the heterotrimeric motor kinesin-2 (REF. 98). It is possible that the motor that is involved depends on the signalling pathway that is employed in each case; the process is phosphatidylinositol-3 kinase (PI3K)-independent in the case of kinesin-1-mediated transport, whereas it is dependent on protein kinase-Cλ

Actin comet tail
A network of actin filaments that is assembled from one end of an intracellular bacterium (such as *Listeria* or *Shigella*) or a cytoplasmic vesicle and that takes on the form of a comet tail. Constant actin polymerization at the surface pushes the bacteria or vesicles through the cytoplasm.

Melanophore
A class of pigment-containing cell that is responsible for the generation of skin and eye colour. The pigment melanin (a tyrosine-polymer) is concentrated in vesicles (melanosomes) that can be translocated in the cell, causing colour change.

Cell cortex
The zone of the cell periphery, directly under the plasma membrane, where the actin cytoskeleton forms a dense meshwork.

CLIP
(Cytoplasmic linker protein). These were originally thought to load endocytic vesicles onto the plus ends of microtubules. CLIPs are part of the microtubule-plus-end-tracking complex (+TIPs) and are associated with the distal ends of microtubules.

Hyphal apex
Hyphae are long, branching filaments that are mostly found in the fungi that form the mycelium, the vegetative network below the ground. Hyphae only grow at the tip, and the hyphal apex contains a growth-related vesicular organelle cluster called Spitzenkörper (tip body).

Rigor mutant
A dominant-negative mutation of the ATP-binding domain in a motor that locks it irreversibly to its cytoskeletal partner.

and RAB4 in kinesin-2-mediated transport. An alternative mechanism which could increase the levels of GLUT4 at the cell surface would be the inhibition of endocytosis. This is apparently achieved by a PI3K- and RAB5-dependent signalling pathway that affects dynein activity, enhancing GLUT4 distribution at the cell membrane[99]. Clearly, more work is required to unravel the network of motor-dependent GLUT4 trafficking. As outlined below, the last step in GLUT4 recycling is linked to myosin Ic, which facilitates the fusion of GLUT4-containing vesicles with the plasma membrane in a PI3K-dependent manner[100].

As observed for the transfer of endocytic vesicles from the cell cortex to microtubules, microtubule-based transport also cooperates with actin-based movements in the exocytic route. As best demonstrated by the melanophore paradigm (BOX 3), a consensus has emerged that proposes that following microtubule transport, myosins drive short-range movements through (or anchor cargoes in) actin-rich regions for local delivery. For example, in the neuronal PC12 cell line, myosin V was proposed to take over exocytic granules from microtubules and to ensure their anchoring[101] and short-range random movements[102] in the actin-rich cell cortex, thereby increasing the probability of membrane docking (FIG. 2). The study on short-range random movements actually reconciled the apparently contradictory views that the actin cytoskeleton functions either as a physical barrier that prevents vesicles reaching the plasma membrane or as tracks that facilitate local vesicle movement and docking. The stabilization and depolymerization of the actin cortex reduced exocytosis, which indicates that just the right amount of cortical actin is necessary to regulate both exocytosis and the replenishment of the releasable pool of vesicles.

Synaptic vesicles undergo repeated cycles of exocytosis and refilling, and they have been extensively studied as carriers that require endocytic recycling for their function. These vesicles have kinesin-1 and myosin V on their surfaces and a direct interaction between them has been reported, which has led to the proposal of a heteromotor complex[103]. However, this observation needs to be independently confirmed. Actin is known to be crucial for synaptic-vesicle recycling[104], and synaptic-vesicle movement at presynaptic terminals that are devoid of microtubules is dependent on myosin Va[105]. Myosin Va on synaptic vesicles directly interacts, in a Ca^{2+}-dependent manner, with plasma membrane syntaxin-1a through its neck domain, and is thereby proposed to regulate stimulated exocytosis through the supply of readily releasable vesicles[106]. Nevertheless, the data linking myosin V with secretion still need to be confirmed *in vivo*.

Syntaxin-1a
A form of SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) protein with a single transmembrane domain that participates in exocytosis. Its C-terminal domain is part of the core SNARE complex, which mediates membrane fusion.

Astrocytes
Star-shaped glial cells in the brain that interact with neurons in multiple ways. They are identified by the expression of glial fibrillary acidic protein (a type of intermediate filament protein) and they outnumber neurons ten to one.

Transfer to the cell surface. Once vesicular cargo has reached the cell periphery by long-range microtubule transport, the final steps of delivery to the plasma membrane, or exocytosis, are finely tuned by a different system that is common to both the endosomal recycling pathway and the secretory pathway.

During the transport of plasma-membrane components from endosomes back to the cell surface, myosin V has a prominent role (FIG. 2). Strong evidence from various cell types links myosin Vb with the recycling of ATP-binding cassette (ABC) transporters[107] and the receptors for transferrin[108], chemokines[109] and acetylcholine[110]. In all cases, motor activity requires RAB11, as recently supported by a permeabilized cell-transport assay[111] and the RAB11-family-interacting protein-2 (FIP2)[112]. For example, the chemokine receptor CXCR2 interacts with both myosin Vb and the RAB11-FIP2 protein in RAB11a-positive vesicles in a manner that is ligand-dependent, and myosin Vb seems important for receptor recycling to the surface[109]. The same complex of myosin Vb and FIP2 might ensure correct inheritance of RAB11a recycling endosomes during cell division[113]. Myosin Vb and RAB11a also have a crucial role in the establishment of apical bile canaliculi in polarized hepatocytes[107]. Most ABC transporters that are required for bile excretion are transported directly from the TGN to the apical membrane. Apparently, myosin Vb and RAB11a are recruited to ABC-positive organelles that also contain transcytotic markers, and they direct these ABC transporters to the surface where they cause differentiation of the site into an apical domain. Therefore, the tripartite complex of myosin V, RAB11 and FIP2 might define a recycling pathway for various cell-surface markers.

Two other myosin V variants have also been implicated in recycling. In astrocytes, myosin Va has been reported in association with recycling endosomes, in which the centripetal movement from the periphery is hormone-triggered and dependent on motor dimerization[114]. In cultured human cells, myosin Vc is thought to contribute to the recycling of the transferrin receptor in a RAB8-dependent manner[115]. Last, a fast and constitutive recycling pathway for surface receptors, which is distinct from the slower recycling-endosome pathway, involves myosin V in complex with three other proteins: hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), actinin-4 and brain-expressed ring-finger protein (BERP)[116].

In addition to myosin V motors, which are dimeric and processive (BOX 1) and therefore can remain associated with actin filaments for several steps, members of the myosin I class of monomeric motors also participate in recycling (FIG. 2). In adipocytes, the return of the glucose transporter GLUT4 from a cortical tubulovesicular organelle to the cell surface after insulin stimulation is linked to myosin Ic[117]. In epithelial cells, myosins Id and Ia function in endosomal recycling[118] and basolateral–apical transport[119] (an epithelium-specific form of a recycling pathway), respectively. In *D. discoideum*, the absence of myosin IB similarly impairs the recycling of plasma-membrane components[120]. Myosins I and V are therefore instrumental in endosome recycling and presumably function primarily in the actin-rich cell cortex near the plasma membrane.

Beyond the similarities between exocytic granules and melanosomes (BOX 3), recent high-throughput strategies have uncovered the composition of various exocytic compartments. Aquaporin-containing vesicles seem to be associated with a broad range of RAB GTPases and myosins, including the recycling-endosome GTPases RAB4, RAB11 and RAB25 and myosin Ic, IIa, IIb, VI and IXb[121]. The specificities of these associations have to be established firmly, but they support other studies that have observed that myosin II is functionally required for the final phase of exocytosis. For example, the fusion of lysosomes with the plasma membrane during wound repair depends on myosin IIb[122]. Also, in chromaffin cells, a mutant regulatory myosin light chain decreases docking and exocytosis[123], and inhibitors of myosin light chain kinase or the myosin ATPase reduce the exocytosis of tear secretory vesicles in lacrimal acinar epithelial cells[124]. In neurons, the interaction of neural cell-adhesion molecule-180 (NCAM180) with the myosin light chain kinase is required to replenish the pool of synaptic vesicles at the active zone after high levels of exocytosis[125], perhaps by regulating presynaptic myosin IIb[126]. Nevertheless, whereas the direct involvement of myosin V has been established beyond doubt, the role of myosin II at the cell periphery is thought to be indirect[127].

Conclusions and outlook

Several features of endosomal trafficking systems are beginning to emerge that need to be followed up in future studies. Both inbound and outbound vesicular carriers can hold several motors on their surface, including those that use different tracks. The direct interaction of microtubule and actin motors and the binding of two motors to a common accessory protein has been shown, but how the signals that start, stop, reverse or continue movement are coordinated on the vesicle is not completely understood. In this respect, the melanosome paradigm (BOX 3) is pointing the way for the design of future studies with other organelles. Knowledge of the motor classes and subtypes that are involved in membrane traffic is still fragmentary. Are the engines really restricted to a limited number of families, as currently available information indicates? Myosin motors, in particular, interact in complex and intriguing ways with the various membrane systems, the microtubule transport machinery and other elements of the cytoskeleton. This is a rich and promising area for future research.

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Competing interests statement

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DATABASES

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