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Linking molecular motors to membrane cargo

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Summary

Three types of motors, myosins, kinesins and cytoplasmic dynein, cooperate to transport intracellular membrane organelles. Transport of each cargo is determined by recruitment of specific sets of motors and their regulation. Targeting of motors to membranes often depends on the formation of large multiprotein assemblies and can be influenced by membrane lipid composition. Motor activity can be regulated by cargo-induced conformational changes such as unfolding or dimerization. The architecture and function of motor: cargo complexes can also be controlled by phosphorylation, calcium signalling, and proteolysis. The complexity of transport systems is further increased by mechanical and functional cross-talk between different types of motors on the same cargo and by participation of the same motor in the movement of different organelles.

Introduction

Two types of cytoskeletal fibers, microtubules and actin filaments, serve as tracks for intracellular transport. These tracks possess intrinsic polarity, as each of them has two structurally and functionally distinct ends, the plus end and the minus end (called the barbed and pointed ends, respectively, in actin filaments). Microtubule-based motors include kinesins, which with a few exceptions move towards the microtubule plus end, and cytoplasmic dynein that moves to the minus end. Actin-based motors, myosins, predominantly walk to the barbed end of the actin filament; the only exception to date is the pointed end-directed motor myosin VI. Eukaryotic genomes typically encode tens of kinesins and myosins with similar motor domains but completely divergent, class-specific, cargo-binding regions while cytoplasmic dynein, when present, is usually represented by a small set of closely related isoforms. Here we describe the emerging principles of motor recruitment and regulation on membrane cargo. For the discussion of the actin-based motors, we focus exclusively on type V and VI unconventional myosins, because much of our current knowledge regarding myosin-dependent organelle transport has come from the study of just these two motors.

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Motor recruitment by multiprotein assemblies

The simplest mode of motor recruitment is through direct binding of the motor to the cytoplasmic portion of transmembrane cargo molecules on transport vesicles/organelles. For example, the yeast type V myosin Myo2 binds to the peroxisome through a direct interaction of its cargo binding domain (CBD) with the transmembrane protein Inp2 [1]. Similarly, kinesin-1 has been suggested to interact directly with the transmembrane amyloid precursor protein (APP) on axonal vesicles [2], while dynein light chains LC8 and TcTex1 were reported to recruit dynein to certain transmembrane receptors [3]. We note, however, that these latter two simple targeting schemes have been disputed, as binding of kinesin-1 to APP transport vesicles might require the c-Jun N-terminal kinase interacting protein (JIP) and/or the Rab GTPase Rab3 [4], while recent structural evidence indicates that LC8 and TcTex1 cannot interact with cargo and dynein at the same time, bringing into question their role as motor-cargo bridging factors [5].

A large body of evidence now points to the importance of multiprotein complexes in the recruitment of motors (Table 1). These can include components of cargo sorting coats such as retromer on endosome-to-TGN carriers [6] which binds to dynactin, as well as the coatomer subunit Ret2, which is required along with the Rab GTPase Ypt11 to recruit Myo2 to Golgi membranes [7]. Similarly, clathrin adaptors can contribute to motor attachment, as AP-1 might link kinesin-3 KIF13A to TGN and endosome-derived carriers [8], while AP-2, together with the endosomal adaptor Dab2, is a part of the complex that links myosin VI to LDL receptor-containing clathrin coated pits and vesicles [9 ″″]. Many motor complexes include compartment-specific Rab GTPases, which frequently associate with their cognate motor through a specialized adaptor protein (Table 1). These adaptors, such as melanophilin (Rab27a) [10–12], optineurin (Rab8) [13], Bicaudal D (Rab6) [14–16], and RILP (Rab7) [17,18], usually recognise their cognate GTPase in its GTP-bound state and thus serve as Rab effectors. By binding simultaneously to the Rab and the motor through non-overlapping domains, these adaptors form a bridge between the activated Rab and the motor. Other tripartite receptor complexes that do not involve a Rab GTPase also exist, such as the complex of Vac8 and Vac17, where the adaptor Vac17 recruits Myo2 to the yeast vacuole by forming a bridge between Vac8 in the membrane and Myo2 [19]. Another type of adaptor is exemplified by GRIP1 and GIPC that interact with kinesin-1 and myosin VI, respectively, and through their PDZ domains associate with the C-termini of transmembrane cargo proteins [20,21]. In addition, multifunctional scaffolding proteins, such as liprins [22] may also be involved in organizing motor complexes and linking them to membranes.

The emerging view of motor: cargo interactions is, therefore, of complex sets of proteins that assemble on the membrane cargo by interacting with each other. For example, the association of kinesin-1 with Trk receptor carriers requires participation of Rab27b, melanophilin-like adaptor Slp1, and multifunctional protein CRMP-2 [23 ″], while targeting of dynein-dynactin to late endosomes depends on Rab7, its effector RILP, lipid-binding protein ORP1L, and βIII spectrin [17] (Table 1). The architecture of such complexes is generally poorly understood and might be complicated by the presence of seemingly redundant interactions. For example, recruitment of myosin Vb to recycling endosomes involves the association of its CBD directly with Rab11 and also with the Rab11 effector FIP2 [24 ″,25]. Similarly, dynein-dynactin can bind to Rab6-positive exocytotic vesicles by interacting directly with Rab6 as well as with the Rab6 effector Bicaudal D [14,15]. In the case of dynein/dynactin, complexity is further enhanced by the important albeit poorly understood roles of Lissencephaly 1 and NUDE/NUDEL proteins, which participate in most dynein-mediated transport processes and might contribute to its membrane targeting [26].

Importantly, in cases where it has been investigated in detail, a relatively small numbers of motors might be involved in pulling cargo at each particular moment [27 ″″], and the

overall number of attached motors might be small (see for review [28]). In contrast, Rabs, adaptors and scaffolds often abundantly decorate the cargo, suggesting that motor recruitment by these molecules might be relatively inefficient.

Control of motor recruitment by the lipids

Membrane compartments can differ in their lipid composition, and these differences appear to influence motor recruitment. Among the lipids that show significant degrees of compartment specificity are phosphoinositides, which can be recognised by various domains in motors or adaptors. For example, the pleckstrin homology (PH) domains of kinesins-3 KIF1A/KIF1Bβ/Unc-104 can interact with phospatidylinositol-4,5-bisphosphate (PIP2) on synaptic vesicles [29], while the PX domain of kinesin-3 KIF16B can bind to phospatidylinositol-3-phosphate (PI(3)P) on early endosomes [30]. A polybasic region, which represents another type of lipid binding domain, is present in the PIP2-interacting CBD of myosin VI [31]. In the case of the myosin Vb-FIP2-Rab11 complex, FIP2's C2A domain might associate with PIP3 in the recycling endosome membrane [32]. Motor recruitment can also be affected by cholesterol, the enrichment of which in late endosomes can regulate the architecture of the dynein-dynactin binding complex through the cholesterol sensor ORP1L [18]. Similarly, the Myo2-Vac17-Vac8 transport complex on the vacuole binds specifically to ergosterol and sphingolipid-rich membrane domains after the palmitoylation of Vac8 [19]. While lipids are unlikely to act as sole targeting factors, they probably contribute to motor recruitment specificity by coincidence detection mechanisms.

Motor "multitasking"

The job of transporting numerous cellular cargos is not distributed evenly among the members of the three motor families. For example, in mammals and flies the majority of microtubule minus end-directed transport processes are carried out by cytoplasmic dynein [26]. Since most known cargos can move along microtubules in both directions, this means that virtually every organelle in animal cells has some kind of dynein receptor. Myosin V is the main "workhorse" in yeast, an actin-centric organism, as it transports at least six distinct membrane compartments (Figure 1). Among animal kinesins, the most ubiquitous and versatile motor is kinesin-1 [33] (Figure 1). Some of these transport pathways require separate adaptors: for example, a complex of the Rho-like GTPase Miro and Milton1/TRAK1 adaptor attaches kinesin-1 to mitochondria [34]. Other adaptors are shared between different pathways: for example, Bicaudal D participates in kinesin-1 and dynein-mediated movements of exocytotic vesicles, nuclei and mRNPs [35–38]. Further, the versatility of a particular motor can be enhanced by alternative splicing: for example, Exon F is required for myosin Va to interact stably with melanophilin [10], while the "long insert" in the tail domain of myosin VI augments its interaction with Dab2 [39].

Coordination of motor activity with cargo binding

To avoid the useless expenditure of energy, many motors may exist in an enzymatically and mechanically inactive form when they are not bound to cargo [40,41]. A classic example is represented by the self-folding of kinesin-1, where in the absence of cargo the tail domain folds back to interact with the motor domain and inhibit its ATPase activity [42 ″]. This autoinhibition is released by the binding of kinesin-1 to two partners: the cargo protein JIP1 (via the kinesin's light chain) and the activator protein FEZ1 (via the kinesin's heavy chain) (Figure 2) [40]. Myosin Va represents another clear example of cargo-driven unfolding/activation, as the binding of melanophilin to the myosin's CBD drives the myosin from its inactive, cargo-free, folded (14S) conformation to its processive, cargo-bound, extended (11S) conformation (Figure 2) [41].

In addition to cargo-dependent unfolding, the activity of some motors is regulated by cargo-dependent dimerization. Motor processivity is generally thought to require the presence of two motor heads working in a coordinated fashion. However, some motors exist as monomers and only dimerize when attached to cargo. One clear example of this is myosin VI: the binding of cargo like Dab2 to myosin VI's CBD converts the soluble monomer to a membrane-associated processive dimer [9 ″″], although dimerization may be further enhanced by subsequent interactions between the two myosin VI heavy chains [43] (Figure 2). Cargo-dependent dimerization via PH domain-dependent interaction with lipids/rafts was also proposed for the kinesin-3 KIF1A [44], although this has recently been disputed [45]. Nevertheless, cargo-dependent control of motor mechanochemistry as a general biological mechanism just makes great sense - it avoids energy waste, prevents free motors from piling up at the ends of cytoskeletal tracks, and promotes motor recycling by diffusion.

Motor cross-talk

It is generally accepted that different motors coexist on the same cargo. Moreover, in microtubule-based transport the ability of organelles to switch their direction of movement is the rule rather than the exception [28,46]. Interestingly, the knockdown of just the plus (or minus) end-directed motor on an organelle can result in a complete block in its bidirectional motility [47 ″″]. There is mounting evidence that different motors on the same cargo can either undergo a tug-of-war or be switched on and off in a coordinated manner [46,48]. Although bidirectional transport can be explained by purely mechanical motor interactions without invoking their physical association [48], detailed studies of motor complexes indicate that certain adaptor molecules can bind to motors of opposite polarity, placing these adaptors in an ideal position to coordinate bidirectional organelle movement. Most of the known examples involve accessory factors for dynein: for example, dynactin can interact with kinesin-2 and affects its processivity [49]. Similarly, Bicaudal D and NUDC, known primarily as dynein co-factors, interact with kinesin-1 and are required for certain kinesin-mediated processes [35,36,50]. HAP-1, an adaptor involved in $GABA_A$ receptor delivery to the synapses [51], interacts with both dynactin and kinesin-1. Syne/nesprin family members, scaffolding proteins in outer nuclear membrane, serve as attachment sites for both dynein and kinesin-1 [38,52 ″]. Physical connections between dynein and kinesins probably reflect the importance of dynein in virtually every transport pathway in animal cells. While the mechanistic reasons for bidirectional transport are not yet entirely clear, it is possible that opposite polarity motors might help each other to avoid roadblocks or activate each other through the generation of mechanical strain [47 ″″]. Furthermore, by linking to various anterograde motors, dynein might be able to hitch a ride to distal cell areas where it cannot be synthesized but needs to function (e.g. the end of an axon or the tip of the cilium).

Motors of the same polarity can also be linked together. A clear example occurs in intraflagellar transport in worms, where two different kinesin-2 motors with distinct intrinsic velocities drive together the same cargo particles, resulting in an intermediate speed of movement [53]. In fact, many organelles such as mitochondria, exocytotic vesicles, and synaptic cargo are probably transported by several redundant kinesins whose cooperation makes transport more robust. Finally, transport of certain cargo, such as pigment granules or recycling endosomes, can be driven by the action of both microtubule and actin-based motors [10,25,54 ″″–56]. Therefore, components of the motor recruitment machinery might facilitate switching between the two cytoskeletal systems. For example, in mouse pigment cells the myosin V adaptor melanophilin accumulates at growing microtubule ends through its interaction with the microtubule plus end-binding protein EB1 [57], while in zebrafish pigment cells it facilitates granule dispersion by regulating dynein [58]. Huntingtin, the protein affected in the neurodegenerative disorder Huntington's disease, interacts not only with dynein, kinesin-1 and dynactin through HAP1, but also with myosin VI through

optineurin, suggesting that it might control switching between different motors and filament systems [59]. In addition, microtubule and actin motors can positively or negatively affect the processivity of each other [60,61].

Control of motor association and function

Motor: cargo interaction must be reversible and its regulation can determine the cargo's final destination. In those instances where a Rab GTPase participates in motor recruitment, the Rab's nucleotide state will be a critical regulatory site. Indeed, manipulation of the expression levels of a Rab27a-specific GAP or GEF dramatically affects myosin V-dependent melanosome distribution in melanocytes [62,63]. Other regulatory mechanisms might involve control of the abundance of the motor and/or cargo adaptor. The clearest example to date is the temporally and spatially controlled degradation of the Myo2 adaptor Vac17 that is required to deposit the vacuole in its correct location during budding [64]. Similarly, the levels of Myo2 receptor Inp2 on peroxisomes are regulated by cell cycle progression and organelle position in the cell [1].

Factors involved in signaling can be an integral part of the motor recruitment complex. For example, motors on frog melanosomes associate with a regulatory subunit of protein kinase A that regulates their movement [65]. Similarly, JIP1, a cargo adaptor of kinesin-1, not only links it to vesicular cargo but also recruits MAPKKK, MAPKK and JNK signaling pathway components that can induce kinesin release (see [40] for review). Another kinase that can exert both local and temporal control of motor dissociation is CAMKII. In neurons, CAMKII activated by elevated cytosolic calcium at post-synaptic sites phosphorylates the CBD of the kinesin-2 KIF17, causing it to dissociate from its adaptor on NMDA receptor vesicles [66 ″″]. CAMKII can also affect synaptic cargo trafficking by inducing the degradation of the scaffolding protein liprin-α1 [67]. During mitosis, CAMKII phosphorylates the CBD of myosin V causing its dissociation from melanosomes [68]. Kinase activity can also potentiate motor: cargo interactions; for example, the Cdk1-dependent phosphorylation of Vac17 increases its affinity for Myo2 [69 ″].

Calcium can also regulate motor complexes directly. For example, calcium influx arrests mitochondria movement in neurons. The key player in this process is the Rho-like GTPase Miro, which participates in kinesin-1 recruitment to mitochondria, and which also contains EF-hand motifs that sense calcium levels. A calcium-induced conformational change in Miro is thought to result in the arrest of mitochondrial movement by causing either the dissociation of kinesin-1 from mitochondria or an inhibition of kinesin-1's interaction with microtubules [70 ″,71 ″]. Calcium influx can also activate organelle movement, as elevated calcium levels in dendritic spines following strong presynaptic input are thought to trigger the movement of AMPA receptor-containing recycling endosomes into the spine by driving the unfolding and activation of myosin Vb [24 ″]. A similar calcium-dependent activation of myosin V might underlie the light-induced movement of pigment granules that drives "pupil" constriction in the fly eye [72].

Finally, a plethora of factors ranging from very small proteins such as Halo [73] to very large proteins such as Huntingtin [59] can affect motor activity, processivity and switching.

Conclusions

Intracellular transport systems face the daunting task of differentially localizing a large number of very diverse cellular structures by using the same set of cytoskeletal tracks in a common cytoplasm. Some organelles have to be distributed evenly, while others must be concentrated in certain regions of the cell or relocated rapidly in response to different stimuli. This complexity in transport requirements places a great number of demands on

motor: cargo interaction - demands for specificity, temporal and spatial regulation, cooperation with other motors, and communication with the machineries that drive the formation and eventual fate of organelles and vesicular carriers. It is becoming increasingly clear that cells deal with these demands by recruiting motors to cargo through large protein complexes that contain small GTPases, specific adaptors, multifunctional scaffolds, and regulatory factors. As more information is becoming available, it appears that in addition to some generic elements, almost every cargo utilizes some specific molecules that participate in motor recruitment. While the catalogue of motor-binding proteins on different organelles is rapidly expanding, understanding how they work together represents a major challenge. In vitro reconstitution experiments using purified components at physiological concentrations will be needed to dissect which components and protein interactions are sufficient as well as necessary for all transport steps [74]. For example, the reconstitution of a Rab- and SNARE-dependent vesicle fusion complex involving 17 recombinant proteins has recently been achieved [75 ″″]. It is likely that similar efforts will be needed to fully understand the complexities of motor recruitment and regulation.

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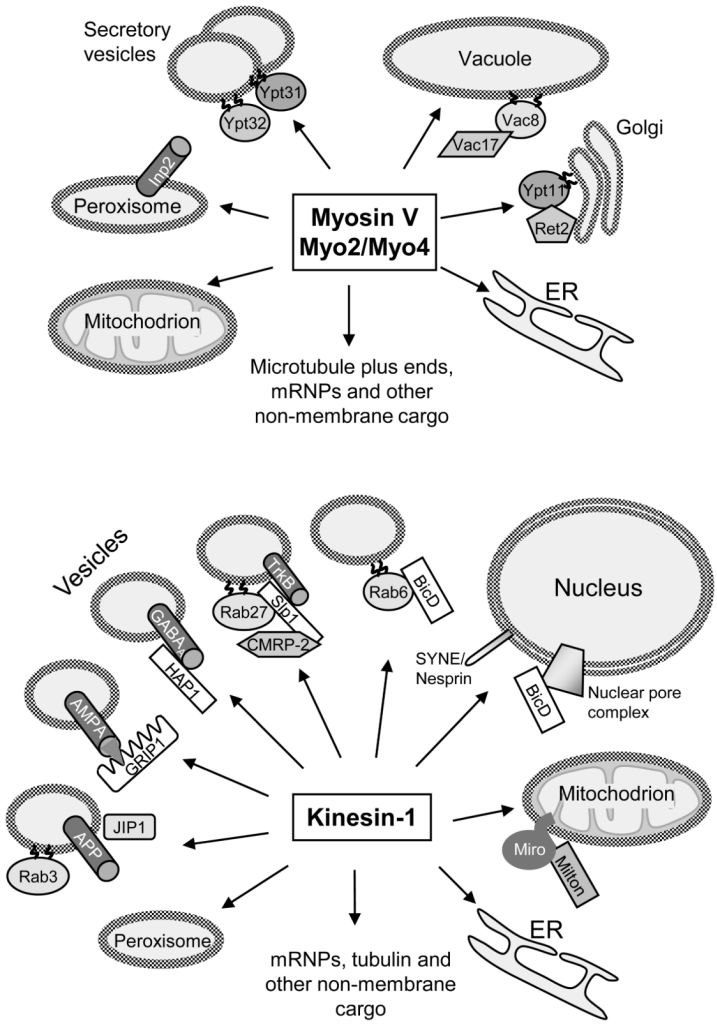

Figure 1. "Multitasking" motors

The scheme illustrates the multiplicity of cargos transported by the two type V myosins (Myo2 and Myo4) in budding yeast and kinesin-1 in mammals. Where known, components of membrane attachment protein complexes and their modes of membrane interaction are indicated. Membrane attachment often depends on lipid anchors, such as geranylgeranyl groups in the case of Rabs (Ypt11, Ypt31/32 and Rab3, Rab6 and Rab27a), and myristoyl and palmitoyl groups for the yeast vacuole protein Vac8 [19]. Transmembrane proteins, such as Inp2 [1], the small GTPase Miro [34], APP [2], and various receptors such as AMPAR [20], $GABA_A$ [51] and TrkB [23 ″] can also serve as a part of the motor attachment complex, often in conjunction with adaptors. Animal nuclei can be linked to microtubule motors through proteins of the Syne/nesprin family, large cytoskeletal linkers that pierce the outer nuclear membrane [52 ″], or through nuclear pore complexes [35]. Compartment-specific motor receptors can attach to unique regions on the surface of the motor's CBD, as has been described for Myo2 [76]. Kinesin-1 also uses different binding sites for different cargo: it is a heterotetramer of two heavy and two light chains, and some adaptors such as Milton, HAP1, and GRIP1 interact with the heavy chains [20,34,51], while others, such as JIP1 and CMRP-2 [23 ″,40], bind to the light chains. Possible competition for the same binding site on the motor has also been described [1], underscoring the need for regulation and coordination in multitasking. For some organelles, such as the ER, motor

receptors are still elusive. In mammalian cells, the transmembrane ER protein kinectin was proposed to act as a kinesin receptor, but its importance was later disputed [33].

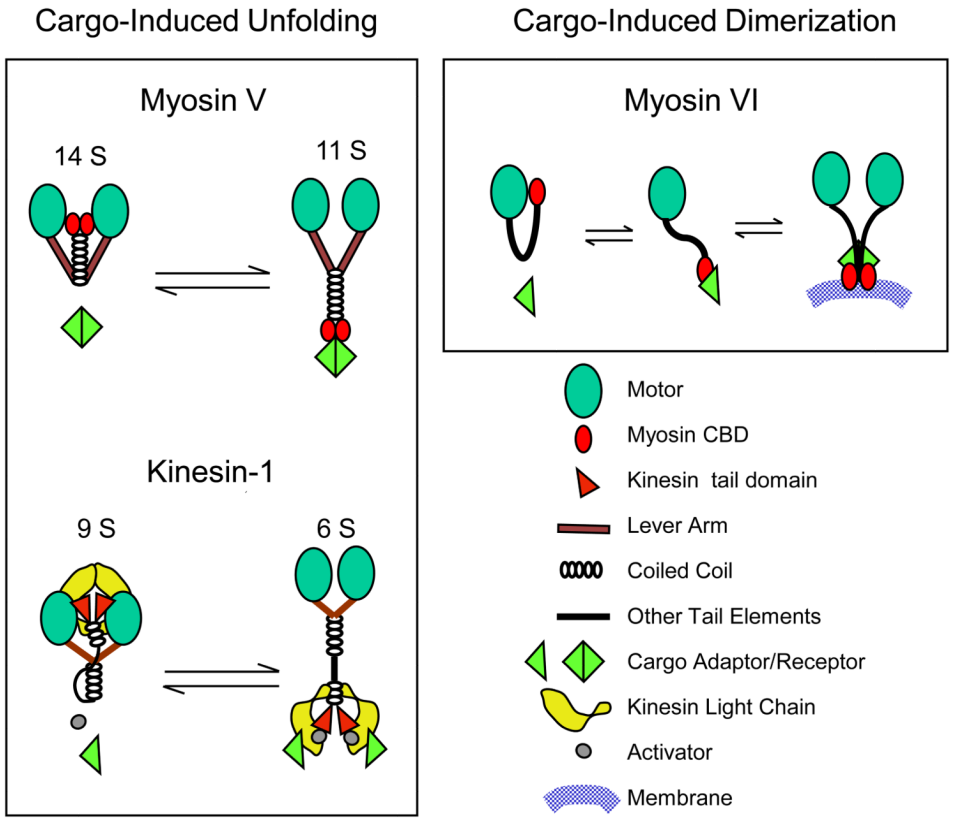

Figure 2. Cargo-dependent regulation of motors

The schemes illustrate the two main mechanisms identified to date where cargo can regulate motor mechanochemistry: cargo-driven unfolding (myosin V and kinesin-1) and cargo-driven dimerization (myosin VI). In the case of myosin V [41] and kinesin-1 [40], these intrinsically dimeric molecules exist in a folded, enzymatically and mechanically quiescent state in the absence of cargo, and in an extended, active state in the presence of cargo. In the case of myosin V, one such unfolding/activating cargo is melanophilin [77], while the unfolding/activation of kinesin-I requires an activator (FEZ1) in addition to a cargo (JIP1) [40]. Whether cargos simply trap the motor kinetically in its extended state or can allosterically induce the extended state remains an important unanswered question. Interestingly, fluorescence resonance energy transfer (FRET) studies showed that in folded kinesin-I, the motor's light chains push the heads far apart, presumably to inhibit motility [40]. Other kinesin family members (e.g. kinesin-2, kinesin-7) are also subject to cargo-dependent unfolding/activation [40]. In the case of myosin VI, a quiescent, folded monomer can be converted to an extended processive dimer through interaction with dimeric cargo [9″″]. Dimerization may be facilitated by subsequent self association of the myosin VI heavy chains through weak coiled coil interactions, as well as by the sensing of membrane lipids by the CBD. Note that the properties of the medial tail of myosin VI (e.g. contribution to dimerization, lever arm length, reverse movement, etc) are currently areas of intense debate (see [43] for review). The cargo-unfolded, monomeric, non-processive version of myosin VI might also support certain cellular functions.

Table 1

Composition of multiprotein motor recruitment complexes on different organelles

The table shows examples of motor complexes with at least two different components in addition to the motor. The table is meant to serve as an illustration and is not comprehensive; the reference list is not complete and is mostly confined to the recent literature that can be used as a source of older references. Cargo proteins are included in the list if they are known to form a part of the motor attachment complex on the membrane. Where known, dynein or dynactin subunits known to be involved in the interaction are indicated. For dynein/dynactin, only a few examples involving Rab proteins are shown, as a multisubunit complex including dynein and dynactin as well as their multiple accessory factors is expected to participate in transport in most if not all cases. Proteins acting as adaptors between small GTPases and motors (Rab effectors) are underlined. Such proteins typically contain separate Rab-binding and motor-interacting domains. Many adaptors are dimers, presumably to support interactions with dimeric, processive motors, and encompass sequences that can form extended coiled coils, probably to facilitate their linker function. In addition to protein-protein interaction domains, some adaptors (FIP2, Slp1) also contain regions potentially involved in lipid binding, such as C2 domains.

Motor	Organelle, cargo	Species	Accessory factors		Ref
			Small GTPase	Adaptors, scaffolds and other proteins	
Myosins					
Myosin Va	Melanosomes	mamm.	Rab27a	melanophilin	[10–12]
Myosin Vb	Recycling endosomes AMPA rec.	mamm	Rab11	FIP2	[24″,25]
Myosin V	Secretory vesicles (rhodopsin)	fly	Rab11	dRip11	[78]
Myosin V Myo2	Vacuole	yeast		Vac8, Vac 17	[19]
Myosin V Myo2	Golgi	yeast	Ypt11	Ret2	[7]
Myosin VI	Post-Golgi vesicles	mamm.	Rab8	Optineurin, Huntingtin	[13,59]
Myosin VI	Clathrin coated structures	mamm.		LDL rec., Dab2 AP2	[9″″,31]
Myosin VI	Clathrin coated structures	mamm.		AMPA rec., AP-2, SAP97	[79]
Myosin VI	Uncoated endocytic vesicles	mamm.		Membrane receptors with PDZ ligand sites (e.g. GLUT1) GIPC	[21]
Kinesins					
Kinesin-1	Mitochondria	mamm.	Miro	Milton/TRAK	[34,70″,71″]
Kinesin-1	TrkB carriers	mamm.	Rab27b	TrkB, Slp1 CRMP-2	[23″]
Kinesin-1	APP carriers	mamm.	Rab3?	APP, JIP1?	[2,4]
Kinesin-1	Neuronal vesicles	mamm.		Membrane receptors with PDZ ligand sites (e.g. AMPA rec) GRIP	[20]
Kinesin-1	$GABA_A$ rec. carriers	mamm.		$GABA_A$ rec., HAP1 Huntingtin	[51]
Kinesin-2 KIF17	NMDA rec. carriers	mamm.		NMDA rec.. Mint1, CASK, MALS	[66″″]
Kinesin-2	Recycling endosomes	mamm.	Rab11	Rab11-FIP5	[55]

Motor	Organelle, cargo	Species	Accessory factors		Ref
			Small GTPase	Adaptors, scaffolds and other proteins	
Kinesin-3 KIF1A/1Bβ	Synaptic vesicles	mamm.	Rab3	DENN/MADD	[80]
Cytoplasmic dynein/dynactin					
Dynein and dynactin	Exocytotic vesicles	mamm.	Rab6	Bicaudal D1/2	[14–16]
p150Glued	Late endosomes	mamm.	Rab7	RILP, ORP1L	[17,18]
Dynein LIC1	Recycling endosomes	mamm.	Rab11a	Rab11-FIP3	[56]