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Cytoskeletal motors and cargo in membrane trafficking: opportunities for high specificity in drug intervention

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Membrane trafficking comprises the directed transport of vesicle and/or organelle cargos to specific locations throughout the cell, and is primarily driven by molecular motors tracking along microtubules and microfilaments. The mechanisms by which specific motor complexes attach to their respective vesicular cargo is of great interest, and is only now starting to be unraveled. The proteins identified as links between the molecular motors and the vesicular cargo are viable drug targets and represent opportunities to regulate small groups of related proteins or even single proteins, such as receptors and transporters, at the cytosolic trafficking level. Ultimately, continued development in this area will lead to greater success in directing endocytosed drugs to the desired intracellular targets, such as the cell nucleus or the basolateral membrane.

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▼ In the field of membrane trafficking, the mechanism by which a molecular motor attaches to its vesicular cargo is of particular interest. Given the complex organization of the eukaryotic cell, with its various components localized in their respective functional locations at the plasma membrane (PM), within the membranes and lumen of organelles, and within the cytosol, highly specific mechanisms have evolved for the attachment of motor proteins to cargos so that they can be shuttled to their required destination. Recently, much progress has been made in this area and an extensive catalog of proteins that link motors to their specific cargo is materializing. In addition, a few general mechanisms for these linkages have emerged.

Owing to their intrinsic specificity, motor–cargo interactions might represent attractive drug targets. It is conceivable that such a therapeutic intervention could result in highly specific regulation of proteins at the trafficking level. In comparison with other drug targets, such as kinases and other proteins involved in signaling pathways that might have a broad range of effects, altering the trafficking of a single protein or a group of functionally related proteins could diminish undesirable side effects and enhance specificity in drug therapy. Additionally, understanding the molecular details of specific motor–cargo interactions might enable one to direct the path a drug takes after it is absorbed into a cell via a vesicular pathway. For example, a drug absorbed via endocytosis could conceivably direct its carrier vesicle straight from the apical membrane to the basolateral membranes, thereby minimizing its exposure to metabolic enzymes or multidrug resistance efflux pumps within an epithelial layer [1].

In this article, current knowledge of specific motor–cargo interactions in vesicle trafficking is reviewed. We provide a list of motor–cargo interactions and related accessory proteins identified in recent studies. The focus of this review is specifically on proteins involved in the most relevant drug-related pathways, which include the excretion or exocytosis pathway and various pathways in endocytosis, such as recycling, degradation, nuclear and transcytosis pathways. The review concludes with a discussion of the implications and possibilities for drug intervention at motor–cargo interactions as they relate to the highly specific regulation of endogenous proteins and the delivery of new and existing drugs.

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Membrane trafficking overview

Cargo vesicles

Vesicles are formed from the PM or intracellular organelles such as the endoplasmic reticulum (ER) or Golgi apparatus. Inside the cell, vesicles, through the action of molecular motors, move at speeds of between 0.1 and 2.0 μm s^{-1} and form ovular or tubular shapes with a long-axis of ~200 nm [2]. The membrane composition of a transported vesicle is similar to that of the plasma or organellar regions from which it originated. However, vesicle membranes are enriched with proteins that often share a common destination and functional characteristics. For example, the amyloid precursor protein (APP) serves as a receptor for the conventional kinesin light chain (KLC) and is localized on vesicles containing β-secretase and presenilin-1, which are believed to cleave APP to form the β-amyloid peptide that is associated with Alzheimer's disease [3]. Similar to the plasma membrane and internal organelles, transport vesicles can have a peripheral matrix or cortical cytoskeleton [4,5].

Motors and the cytoskeleton

Vesicles travel along cytoskeletal filaments with the aid of molecular motors, which hydrolyze ATP to power their movement. Members of the kinesin and dynein families move along microtubules for long-range delivery of vesicles, whereas myosin family members move along actin microfilaments near membrane layers during vesicle budding and fusion processes. Microtubules and microfilaments are polarized owing to asymmetry in their monomeric tubulin or actin constituents, respectively. With regard to motor activity, this polarization results in unidirectional movement towards the rapidly growing (plus) end or towards the slowly growing (minus) end of the filament. Most members of the kinesin and myosin families move towards the plus end, whereas all known dyneins move towards the minus end. Additionally, motors nearly always comprise multimeric complexes consisting of heavy chains and, depending on the motor, a combination of intermediate and light chains. For example, members of the conventional kinesin (often refered to as kinesin I or simply kinesin) subfamily are tetrameric complexes containing two heavy-chain motor domains and two light-chain domains. Additional adaptor or accessory proteins are also often required. For a full review of the kinesin, dynein and myosin motor proteins, the reader is referred to Refs [6–8].

Exocytosis and endocytosis

Trafficking pathways with potential application to drug discovery are the exocytosis (excretion) and endocytosis pathways, with the exocytosis pathway being of particular interest. Based on the current knowledge of motor–cargo interactions, it would be feasible to begin screening drugs that mediate the interactions of motor molecules with specific cargos. Receptors and transporters are trafficked through the exocytosis pathway to their destination at the PM or to the membrane of other internal organelles, such as the nucleus or lysosomes. Therefore, regulation of these proteins at the trafficking level is feasible in these cases where motor–cargo interactions have already been elucidated. For example, the activity of the multidrug resistance 1 efflux transporter (MDR1) P-glycoprotein (Pgp) might be reduced by drug intervention at the trafficking level. The feasibility of this approach is exemplified by the effects observed in Dubin–Johnson syndrome, where a mutation in multidrug resistance-associated protein 2 (MRP2) causes mis-sorting and ER accumulation of the protein. The result is a low level of expression of MRP2 at the PM and an increase in the levels of intracellular bile acid conjugates, leading to hyperbilirubinemia [9]. Recently, intracellular pools of Pgp were shown to traffic rapidly to the PM after treatment with the chemotherapeutic agent mitomycin C, despite a decrease in MDR1 mRNA levels [10]. This implies that protein might traffick from the trans-Golgi network (TGN) to an intracellular store before the PM. Another example is the ΔF508 isoform of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, which is not appropriately trafficked to the PM, resulting in the CF phenotype [11]. A recent study showed that treatment with doxorubicin, a cancer chemotherapeutic agent, increases the PM expression of this ΔF508 isoform without increasing transcription in Madin-Darby canine kidney cells, suggesting that regulation by doxorubicin at the trafficking level is responsible for the observed increase in protein expression [11]. As the authors of this study state, the drug-sensitive trafficking mechanisms of the ABC transporters CFTR and Pgp might be similar. Therefore, the elucidation of these trafficking events is crucial for the total understanding of drug disposition [10].

The endocytosis pathway is also of interest for future applications of motor–cargo research. Pathways that involve vesicle formation at the PM are potential entry points for drugs, and serve as models for how intervention of motor–cargo interactions might improve the delivery and specific intracellular targeting of drugs. Pathways that could deliver drugs to the nucleus or the basolateral membrane are of particular interest because drugs such as anticancer agents often target transcription and therefore need to enter the nuclear compartment [2]. Drug delivery currently relies heavily on the physicochemical properties of the drug and its delivery system to determine subcellular localization. Unfortunately, this reliance often results in

the untargeted delivery of the drug to the cytosol or other cellular compartments. For example, Rapoport and colleagues used a micellar delivery system for delivery of ruboxyl, a derivative of the anticancer drug doxorubicin, to the cell nucleus in cultured A2780/ADR cells. The authors attribute the selective localization of ruboxyl to its affinity for the micellar membrane, which is intermediate between the affinity of this drug for intracellular membranes and DNA [1]. The authors correctly point out the benefit of drug delivery through endocytosis as a way to prevent efflux through multidrug resistance transporters.

Various gene therapy approaches, including antisense, triplex and RNA interference, have shown promise in gene regulation. However, nucleic acid delivery to the cell is a rate-limiting step in the development of a therapeutic strategy [12]. The herpes simplex virus 1 (HSV1) protein U(L)34 interacts with dynein intermediate chain IC-1a [13]. This protein appears to contain a nuclear targeting signal, which might be a mechanism by which virus particles reach the cell nucleus [13]. Further elucidation of the motor–cargo interactions involved in the trafficking signaling of this protein should enhance its potential use in drug delivery.

Transcytosis of drugs through the epithelial layer is often desirable. Although many drugs can be modified to increase their absorption into the epithelial layer, they might be degraded, immediately exported via MDR transporters or trapped within intracellular vesicles (such as acidic vesicles in the case of positively charged molecules [2]), thus never reaching the desired destination (e.g. systemic circulation). Control of trafficking might enable endocytosed vesicles to carry drugs to the basolateral membrane, therefore bypassing metabolic enzymes and MDR exporters.

Although the kinesin and dynein motor families are involved in parts of both the exo- and endocytosis pathways, they each serve primary roles with respect to their direction of transport. In general, kinesins are important motors in the exocytosis pathway because they move relevant cargo in an anterograde direction from intracellular regions to the PM and to other organelles. Dynein is highly relevant in the endocytosis pathway because of its retrograde movement of endosomes into the cell interior. Myosin is involved in both pathways near the cell membrane, after vesicle formation in endocytosis and vesicle fusion in exocytosis. Transitions between microtubule and microfilament movements might be facilitated by the known interactions of myosin with kinesin [14] and dynein [15]. Figure 1 provides a representation of the pathways relevant in this review. The reader is referred to Ref. [16] for further details on both the exocytosis and endocytosis pathways.

Motor–cargo attachment

General mechanisms

The attachment of vesicular cargo to a motor protein is an interaction between the motor and a cargo-bound receptor, usually through an accessory or adaptor protein or complex [17]. Some clarification is warranted for distinguishing between motors, receptors and adaptors because all of these can be multimeric complexes. Although the motor is nearly always a multimeric complex, for simplification and for understanding the motor–cargo attachment mechanisms discussed in this review, the heavy-chain motor domain (the ATPase that binds to the cytoskeleton and generates motion) can be considered as the primary defining component of the motor. This view allows one to better understand that a single heavy chain (or more often a heavy-chain dimer) can dock directly with cargo or it can bind to various other adaptors such as intermediate chains, light chains or numerous other non-motor proteins that are required for heavy-chain function and/or cargo binding. The receptor, often specific to the cargo, is directly attached to the vesicle. Receptors are generally either transmembrane proteins or scaffold proteins [17]. Adaptors are any protein that links the motor to the receptor, including single proteins and large protein complexes. Motor–cargo attachment mechanisms are varied, although common mechanisms do exist. Figure 2 summarizes these general attachment mechanisms. In general, specificity in cargo binding exists at all three links in the attachment chain. For example, most motors have a limited set of cargos that can be transported. Similarly, receptors are specific to the type of vesicle (the types of biomolecules contained within the vesicle) to which they attach. Finally, adaptors have limited motor and receptor binding partners. Therefore, intervention with any one link in the chain can inhibit the transport of specific cargo without affecting additional interactions the other links might have.

Two recent reviews provide a comprehensive summary of the elucidated mechanisms of attachment for various motors and cargo [17,18]. Other highly relevant information can be found in Refs [19–21]. Following the publication of these reports, several new attachment mechanisms have been determined for a variety of motors and receptors, which illustrates the rapid advances in this research area. The following sections provide an overview of the attachment mechanisms identified within the past year, and Table 1 summarizes the information presented in the text.

New discoveries in the kinesin family

Although it was proposed originally that conventional kinesin heavy chain (KHC) binds indiscriminately, whereas

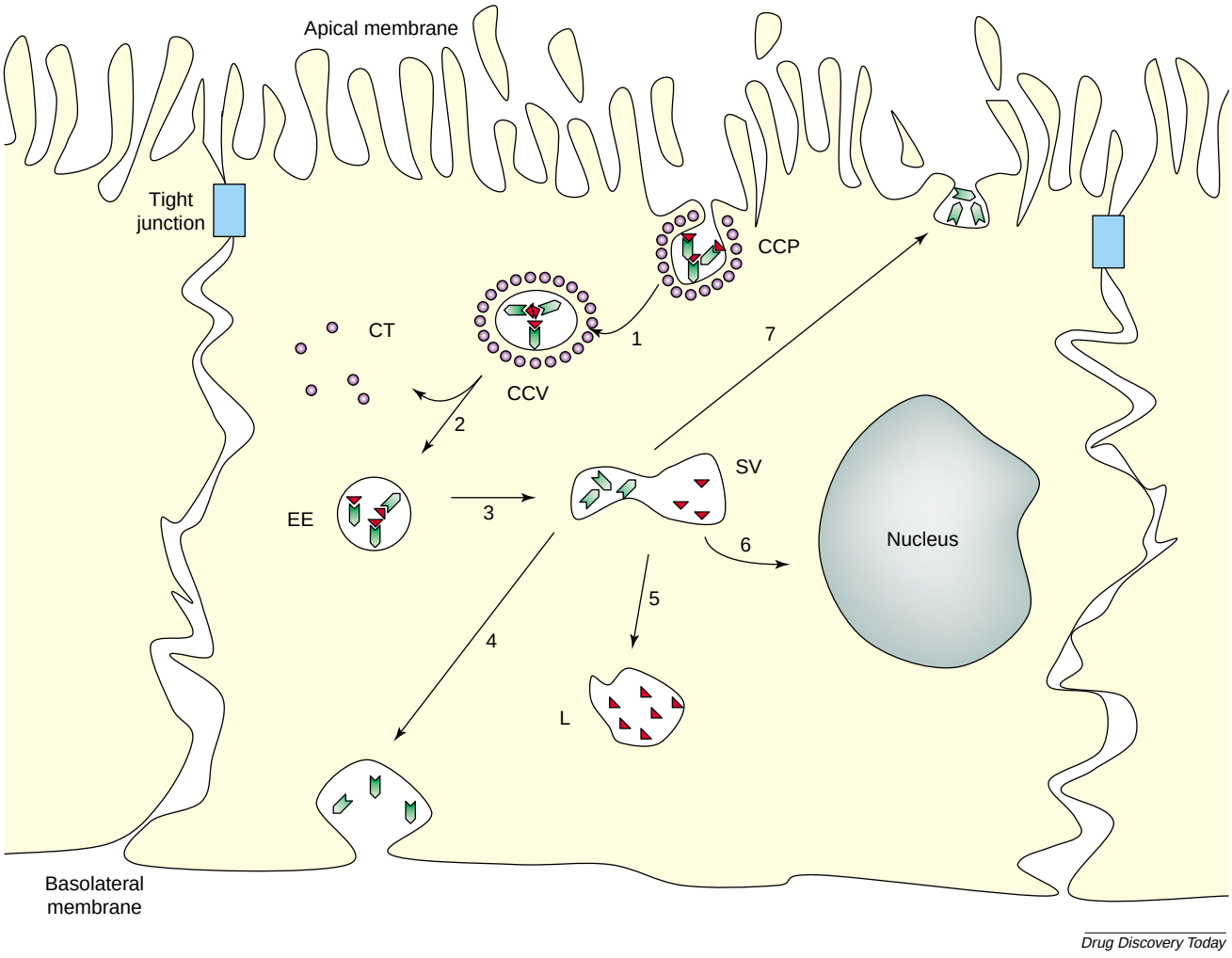

Figure 1. Representative pathways that are involved in membrane trafficking within an epithelial cell. Interactions between cytoskeletal motors and vesicle cargo determine which pathway the vesicle will take. In receptor-mediated endocytosis, ligand (red)-bound receptors (green) are localized in clathrin-coated pits followed by vesicle invagination and scission from the plasma membrane (1). Clathrin triskelions (purple) dissociate from the outer vesicle membrane (2), and lowered intravesicular pH causes some ligand dissociation from receptors. Receptors, ligands and other cargo are then sorted in sorting vesicles (3) where new vesicles are packaged and directed along various pathways to the basolateral membrane (4), lysosomes (5), nucleus (6) or back to the apical membrane (7). In exocytosis or excretion, similar pathways are taken by newly formed proteins that must arrive at their appropriate destination at the plasma membrane (4,7) or other internal organelles (5,6) for proper functioning. Motor–cargo interactions along the various pathways represent potential drug targets. Drug-induced alteration of these interactions might enable us to direct cargo along these pathways to desirable destinations (e.g. an endocytosed drug directed to the nucleus or an endogenous multidrug resistance transporter directed away from the plasma membrane). Abbreviations: CCP, clathrin-coated pit; CCV, clathrin-coated vesicle; CT, clathrin triskelion; EE, early endosome; L, lysosome; SV, sorting vesicle.

KLC isoforms bind to specific cargo receptors [17,21], subsequent findings suggest that KHC also displays significant variability in its cargo. Conventional kinesin KIF5 (kinesin family protein) was recently shown to interact with the α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor subunit GluR2, and GRIP1, a GluR2-interacting protein [22]. The researchers concluded that GRIP1 acts as an adaptor, linking the motor and the receptor. This motor–cargo interaction represents the concept of a 'smart motor' in neuronal trafficking [22]. It is unclear how motor–cargo complexes are directed to either the distal dendrite or axon because both are composed of microtubules with plus ends directed away from the cell body. The authors proposed that cargo that is bound to a smart motor establishes a directional signal within the resulting motor–cargo complex, thereby directing it to the appropriate pathway [22]. In a separate study, the neuron-specific motor KIF1A was found to link to Liprin-α/SYD-2, another

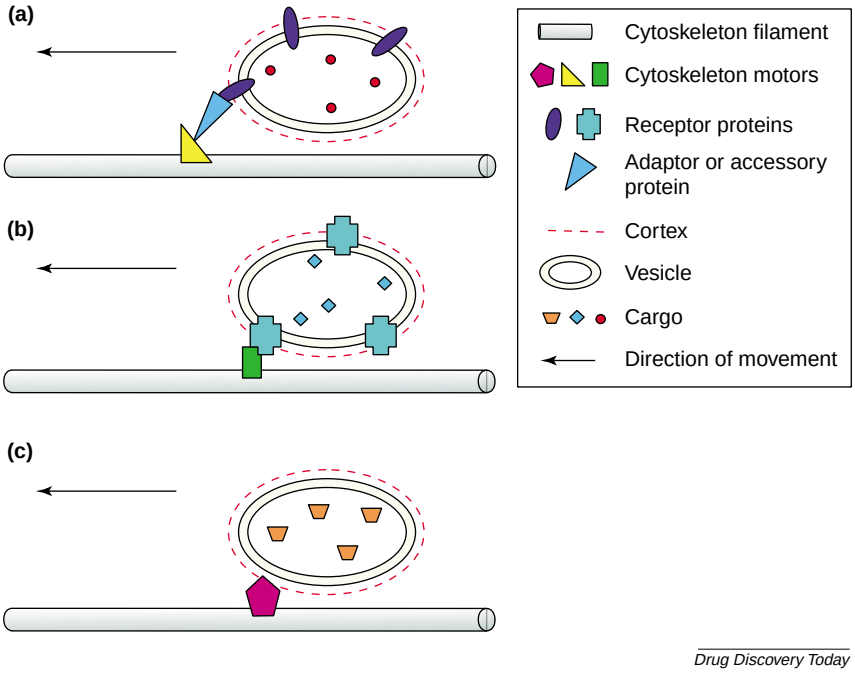

Figure 2. Schematic of three general motor–cargo attachment mechanisms: **(a)** a commonly observed mechanism whereby an adaptor protein or complex links the motor to the vesicle-bound receptor; **(b)** the motor binds directly to the vesicle-bound receptor; and **(c)** the motor binds directly to the protein cortex of the vesicle or organelle. Motors are shown with various symbols to illustrate that these general mechanisms are feasible with kinesin, dynein and myosin. Symbols for cargo (including receptors) are shown as homogeneous within a given vesicle to illustrate that vesicles are enriched with functionally related proteins. Finally, motors are nearly always complexes of many proteins whereas adaptors and receptors can be either complexes or single proteins.

scaffold protein for AMPA glutamate receptors [23]. This study also reported interactions with GRIP1, and the authors proposed that KIF1A also accepts cargo destined for dendrites. However, more work is needed to confirm these hypotheses.

In another study, the conventional kinesin heavy chain KIF5B was shown to interact with the synaptosome-associated proteins SNAP-23 and SNAP-25 [24]. SNAP-23 is a presynaptic t-SNARE protein involved in the recognition of excreted synaptic vesicles. SNAP-25 is hypothesized to have a similar function. These findings support the idea that synaptic complexes are assembled in the cell body and directed to their destination at the nerve terminal via axonal transport. In this scenario, the SNAP proteins act as trafficking receptors linked directly to the KHC. The role of other proteins with possible involvement, such as SNAP-related proteins, synaptobrevin and syntaxin, is currently unclear [24]. These workers also studied the trafficking of the HSV protein US11 and identified an interaction with the conventional KHC. This interaction is believed to be important for viral entry and trafficking within the neuron. These findings are highly significant because an understanding of the mechanism by which US11 travels to the nucleus could lead to nuclear-targeting drug strategies [25].

Finally, a study by Conforti and colleagues investigated the differences in expression levels of two isoform splice variants of the same gene, Kif1Bα and Kif1Bβ, in a mouse model of amyotrophic lateral sclerosis. Of particular importance is that these two isoforms, varying in their C-terminal peptide domains, bind significantly different cargos. The α isoform transports mitochondria, whereas the β isoform transports synaptic vesicle precursors [26], thus further illustrating the available diversity and specificity of cargo binding.

Kinesin plays an important role in neuronal trafficking owing to its role in transporting axonal cargo over relatively long distances. Dynein motors are also important in this process. Links between trafficking dysfunction and neurological disorders have been identified in diseases such as the Charcot-Marie-Tooth disease type 2A (a kinesin-related disorder) and lissencephaly (linked to dynein function) [27]. Further work in characterizing motor–cargo interactions for improved drug targeting strategies will perhaps help to correct these and other trafficking-related diseases.

New discoveries in the dynein family

The large number of proteins that interact with dynein, including its light, intermediate and heavy chains, in addition to the massive dynactin complex containing ~15 proteins, provides dynein with a diversity in cargo specificity. Dynein requires dynactin to associate with membranes; however, dynactin alone cannot associate with membranes. It is currently postulated that the actin-related protein 1 (ARP-1) subunit of dynactin binds to spectrin associated with organellar membranes such as the TGN [18]. Kamal and Goldstein proposed that dynactin binds to cortical components of vesicles, whereas the dynein subunits interact directly with cargo such as rhodopsin and the neurotrophin receptor trkA [17].

The function of dynein in the exocytosis pathway is to transport vesicular cargo from the intermediate compartment

to the Golgi apparatus, in addition to moving vesicles and organelles, such as lysosomes and endosomes, into the cell interior [17]. Dynein is also a regulator of kinesin-driven transport from the TGN, demonstrated in the bi-directional movement of vesicles trafficking to the PM. Bicaudal-D1 (BICD1), an adaptor that binds to the membrane-bound Rab6a and to the dynein–dynactin complex, regulates dynein motor function and the consequent retrograde activity of vesicles traveling from the TGN to the PM [28]. Hoogenraad and colleagues previously determined that BICD2 regulates the dynein–dynactin interaction (through direct binding to dynamitin) and, therefore, dynein motor function [29]. The Matanis study illustrates that BICD1 facilitates and regulates the dynein association with Rab6a-positive membranes by slowing the rate (through increased bi-directional movements) at which these membranes are transported towards the PM.

Table 1. Motor–cargo interactions published since January 2002[a,b]

Motor	Subunit	Adaptor	Receptor	Refs
Dynein	Dynactin	BICD1	Rab6a	[28]
Kinesin	KHC	–	SNAP-25 and SNAP-23	[24]
Kinesin	KHC	–	US11 (viral HSV)	[25]
Kinesin	KIF5	GRIP1	GluR2	[22]
Myosin II	UNC-54, MYO-1	–	ITR-1	[49]
Myosin Va	–	Melanophilin (SLAC2-A)	Rab27a	[33] [34]
Myosin Vb	–	Rab11-FIP2	Rab11a	[38]
Myosin VI	–	DOC-2/DAB2	AP-2–clathrin	[43,44]
Myosin VI	–	SAP97	GluR1	[45]

[a]Only motor–cargo studies that confirmed transport via the associated motor are listed. Several studies were published listing motor linkages to adaptor or scaffold proteins without direct confirmation of trafficking via the associated motor (see text). These studies are not listed in the table.
[b]Abbreviations: AP-2, adaptor protein-2; BICD1, bicaudal-D1; DOC-2/DAB-2, Disabled-2; GluR1, AMPA glutamate receptor subunit 1; GRIP1, GluR2-interacting protein; ITR-1, *Caenorhabtidis elegans* inositol (1,4,5) trisphosphate receptor; Rab11-FIP2, Rab-11 family interacting protein-2; SAP97, synapse-associated protein 97; SLAC2-A, Slp homologue lacking C2 domains-A; SNAP, synaptosome-associated protein; UNC-54 and MYO-1, *C. elegans* myosin II heavy chains; US11, herpes simplex virus (HSV) RNA-binding protein.

Several studies have revealed dynein light chain (DLC) interactions with the cytosolic domains of membrane proteins, suggesting a possible motor–cargo adaptor linkage. However, these studies did not report a prior or subsequent linkage to the dynein motor. In one study, Schwarzer and colleagues reported that the voltage-dependent anion-selective channel (VDAC) interacts with the DLC Tctex-1 [30]. However, a complex containing the channel, DLC and the dynein motor was not identified. In a study by Mueller and colleagues, the cytoplasmic domain of the poliovirus receptor CD155 was found to bind strongly with Tctex-1 [31]. Although this strongly suggests a mechanism for retrograde axonal transport of the polio virus via CD155, further studies will be required to determine if dynein eventually joins the CD155–Tctex-1 complex. In another study, the cytoplasmic domain of the transforming growth factor β receptor (TGFβR) was reported to bind with LC7, a DLC [32]. Further analysis revealed that the interaction is not involved in TGFβR trafficking but it is instead a regulation mechanism to prevent LC7 from associating with the dynein intermediate chain (DIC) and ultimately other cargo [32]. This study illustrates that heavy-chain recruitment to a complex of interacting non-heavy-chain motor components and other proteins is not guaranteed.

New discoveries in the myosin family

Myosin V is the primary myosin responsible for vesicle trafficking and, as is typical with most motor proteins, cargo specificity is localized at the C-terminal domain [18]. Two independent studies recently identified that melanophilin (SLAC2-A) is an adaptor that links myosin Va with Rab27A, a membrane-bound rab GTP-binding protein localized on melanosomes [33,34]. A previous study had suggested that myosin Va and Rab27a interact [35] but it was not until recently that melanophilin was identified as the adaptor. Another interesting finding in this interaction is that a splice variant of myosin Va containing exon F is required for attachment to melanophilin and the consequent localization to melanosomes [36]. Myosin Va is known to have at least three other tissue-specific splice variants that affect the cargo-binding C-terminus, and these will probably be found to determine adaptor and/or cargo specificity [36].

The importance of myosin Vb as a motor that allows endocytosed cargo to complete the recycling pathway back to the PM has been demonstrated [37]. This study indicated that myosin Vb interacts directly with Rab11a. Hales and colleagues [38] recently demonstrated that Rab11a is linked to the myosin motor through Rab11–FIP2 (Rab11 family interacting protein 2). They noted that a possible explanation for the dual ability of myosin Vb to bind Rab11a can be explained by the fact that direct binding only occured when GTP was bound to Rab11a, whereas the

Box 1. The glutamate receptor: a model for targeting the motor–cargo interaction

Glutamate receptor-mediated excitotoxicity is a proposed mechanism for neurodegeneration in amyotrophic lateral sclerosis (ALS), Huntington's disease and other progressive neurological disorders [50]. Multiple glutamate receptor subunits exist, and these subunits combine to form receptor subtypes selective for *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainate (KA). ALS is linked primarily to over-activity of the AMPA receptor subtype, while Huntington's disease is associated with the NMDA and KA receptor subtypes. A promising means of protecting against excitotoxic effects and potentially impeding or even halting the progression of these diseases is to block glutamate receptor activity with antagonists or to downregulate their cell surface expression [50,51]. Although some selective agents are now available, antagonist drug design is a significant challenge because it is difficult to block one receptor subtype without affecting other receptor subtypes owing to the diversity of receptor composition and the similarity in the structure of substrates [52,53]. Consequently, a means to downregulate specific glutamate receptor subunits, and therefore subtypes, is desirable.

As discussed in the text and in Table 1, motor–cargo interactions for the AMPA glutamate receptor subunits, GluR1 and GluR2, have been studied recently. Drugs designed to specifically target and interfere with the GluR1–SAP97 and GluR2–GRIP1 interactions would probably result in a decreased cell surface expression of these two subunits and consequently the AMPA receptor subtype. Other recent work also suggests that the various glutamate receptor subunits interact to varying degrees with different adaptor or scaffold proteins [54]. Therefore, no or minimal effects on other glutamate receptor subunits would be expected. Specifically targeting these interactions, as opposed to the KIF5–GRIP1 or myosin VI–SAP97 interactions, could further reduce the potential for side effects that could result from the motor–adaptor interactions in other cellular processes. The hypothesized result would be a reduced level of AMPA receptor cell surface localization and less susceptibility for excitotoxicity. The ability to target specific AMPA subunits could conceivably reduce necrosis in ALS patients without sacrificing the signaling of other glutamate receptors. Much work still needs to be done to evaluate the specificity of these interactions (i.e. the GluR1–SAP97 and GluR2–GRIP1 interactions) and the physiological effects of blocking them. However, this example demonstrates the possibilities for motor–cargo interactions to serve as viable alternative drug targets.

adaptor configuration with Rab11–FIP2 could occur regardless of the GTP-bound state of Rab11a [38]. In this study, mutations in Rab11–FIP2 caused mis-sorting of IgA and transferrin in MDCK and HeLa cells, respectively, showing that the adaptor is a crucial component in the interaction [38]. The binding between Rab11–FIP and Rab11 was further characterized and it was demonstrated that the interaction occurs in a post-rab11 endosome [39]. Another recent study showed that myosin Vb is important in recycling of the acetylcholine muscarinic M_4 receptor, although the results did not clearly indicate whether an adaptor might be involved in linking the motor to Rab11a-associated endosomes [40].

Myosin VI and the newly discovered myosin IXb are the only two known minus end-directed myosin motors [41]. Although little is known about myosin IXb, several studies have been performed on myosin VI, and this protein is believed to be important in endocytosis, moving newly-formed vesicles away from the PM [42]. It was recently shown that myosin VI interacts with the signaling protein Disabled 2 (Dab2) [43,44]. Further biochemical evidence suggests that Dab2 might be an adaptor for linking myosin VI with AP-2 in clathrin-coated pits [44]. Finally, a study by Wu and colleagues identified that myosin VI binds SAP97, a synapse-associated protein, in rat brain extracts [45]. Biochemical methods also illustrated that these two proteins form a ternary complex with GluR1, a subunit of the AMPA glutamate receptor, suggesting that SAP97 could be an adaptor for the motor–receptor interaction [45]. At what stage of the endocytosis and recycling of GluR1-containing vesicles this interaction occurs is not known. Interestingly, myosin VI and SAP97 did not interact when tested in the Caco-2 intestinal cell line, suggesting that the interaction is regulated in a tissue-specific manner.

Motor–motor interactions

Motor–motor interactions have been demonstrated between all three motor families. Myosin V was shown to interact directly with both the KHC and DLC8 [15]. These interactions possibly represent a transitioning mechanism between the microtubule and microfilament cytoskeletal systems during anterograde (myosin–kinesin) or retrograde (myosin–dynein) transport. However, further studies will be required to identify the significance of the interactions. Of particular interest is the interaction between dynein and kinesin. A subunit of the dynactin complex, p150Glued, was shown to interact with KIF3, a heterotrimeric motor complex of the kinesin II subfamily, through the KIF3 non-motor subunit XKAP (a *Xenopus* kinesin-associated protein) on melanosomes in oocytes [46]. This finding could represent a means of transporting dynein to MT plus ends (or kinesin to MT minus ends). Alternatively, it might

represent a multifunctional complex capable of moving along both cytoskeletal systems, thus implying that the observed bi-directional movement of vesicles in the exocytosis pathway is due to bi-functional motors as opposed to the coincident attachment of both kinesin and dynein motors to the same vesicle.

Implications for drug discovery and delivery

Membrane trafficking offers an exciting novel target for therapeutic intervention. Control of the cellular destination of both endogenous biomolecules and drugs absorbed through endocytotic mechanisms is an attractive concept with increasing possibilities. The continued discovery of proteins involved in motor–cargo attachment lengthens the list of potential drug targets. Owing to the specificity of some of the interactions already elucidated, it can be envisioned that therapies that interfere with the attachments of motors to specific cargo could be implemented with minimal cross-reactivity and side effects. As a summary and example of how the ideas and experimental results discussed in this review could be applied, Box 1 illustrates the potential to alter the progression of neurodegenerative diseases by regulating the motor–cargo interactions that are responsible for the cell surface expression of glutamate receptors through drug intervention.

Interactions of proteins with motors do not necessarily indicate a trafficking mechanism for the interacting protein. Similarly, *in vivo* studies sometimes reveal more complexity and less specificity in the trafficking mechanisms and interactions originally discovered by *in vitro* methods. An example is a study performed by Setou and colleagues, where the kinesin KIF17 was found through several *in vitro* methods to indirectly bind and transport the NR2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor through attachment to the scaffold adaptor Mint1 (mLin-10) [47]. However, a more recent study using Mint1 knockout mice demonstrated normal functioning of the NMDA receptor, thus suggesting that the previous *in vitro* results might not reflect a physiologically relevant interaction [48]. These observations underline the essential requirement of thorough assessments of motor–cargo interactions if these are to be useful drug targets.

As the understanding of the motor–cargo attachment mechanisms develop, it is possible that many of the trafficking pathways could be controlled to the point that a drug could be directed to very specific locations within the cell. Although this is theoretically achievable and consistent with much of the data that has been generated thus far, this will take a considerably higher level of understanding of not only the individual motor–cargo interactions but also of the numerous protein–protein interactions that take place within the various pathways as vesicles and their cargoes are sorted. The key to effective therapeutic intervention is first to identify the motor–cargo or motor–adaptor–cargo interactions responsible for specific transport and, second, to design drugs to alter these interactions in a specific fashion.

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