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Unconventional functions of microtubule motors

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Abstract

With the functional characterization of proteins advancing at fast pace, the notion that one protein performs different functions – often with no relation to each other - emerges as a novel principle of how cells work. Molecular motors are no exception to this new development. Here, we provide an account on recent findings revealing that microtubule motors are multifunctional proteins that regulate many cellular processes, in addition to their main function in transport. Some of these functions rely on their motor activity, but others are independent of it. Of the first category, we focus on the role of microtubule motors in organelle biogenesis, and in the remodeling of the cytoskeleton, especially through the regulation of microtubule dynamics. Of the second category, we discuss the function of microtubule motors as static anchors of the cargo at the destination, and their participation in regulating signaling cascades by modulating interactions between signaling proteins, including transcription factors. We also review atypical forms of transport, such as the cytoplasmic streaming in the oocyte, and the movement of cargo by microtubule fluctuations. Our goal is to provide an overview of these unexpected functions of microtubule motors, and to incite future research in this expanding field.

Keywords

Molecular motors; Kinesin; Cytoplasmic dynein; Cytoskeleton; Microtubule dynamics; Cell signaling

Introduction

The genomic and proteomic revolution during the recent years has led to the identification of almost the complete set of proteins that is expressed in cells. While today we no longer think of proteins as the sole effectors of cellular functions, they still are the best-characterized executors of cellular activities. With the systematic exploration of protein function under way, the notion that one protein performs different functions – often with no relation to each other - emerges as a novel principle on how cells operate (see, for example [1]). Recent findings clearly show that molecular motors are no exception to this trend. While molecular motors have been extensively characterized as “machines” that power intracellular transport of cargo by hydrolyzing ATP [2-6], reports over the years indicate that they are involved in additional functions, which may either rely on, or be independent

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of, their motor activity [7-10]. The best-studied example of the first category is the ability of some kinesins to depolymerize microtubules, a process important for regulation of the cytoskeleton. By contrast, other kinesins stabilize, or promote elongation of, microtubules. Modifying cell and organelle shape by generating tension are also unconventional functions of motors that require their motor activity. Examples of motor independent functions include the direct participation of kinesins in cell signaling, e.g., by blocking the interactions between transcription factors, or by functioning as scaffolds that recruit active kinases to their substrates, thus facilitating their phosphorylation. Other examples are those where the molecular motors - kinesins and cytoplasmic dynein - anchor protein and mRNA complexes to the cytoskeleton or to the plasma membrane. In this review, we will expand on the idea of microtubule motors as multifunctional proteins that regulate multiple cellular processes, in addition to their main function in transport. We will discuss a few examples that make the point, focusing not only on firmly established unconventional functions, but also on emerging, less characterized, but thought-provoking functions. Myosin motors, generating actin-dependent motility, also accomplish unconventional functions, but these are not covered by this review. In fact, the term “unconventional myosins” has been adopted by the myosin field long ago [11, 12], to distinguish them from the “conventional” class II myosins, which form filaments and are present in the traditional acto-myosin complexes that perform muscle contraction or cytokinesis [13]. Before exploring the unconventional functions of microtubule motors, we will briefly summarize what is currently accepted as their conventional function.

The conventional function of microtubule motors

The major function of microtubule motors in interphase cells is to power transport of cargo between intracellular compartments and locations, a task that is particularly stringent in neurons [2, 14]. This function relies on the motor's ability to bind to a large variety of cargo [2, 5, 9, 15-17], and to translocate – by hydrolyzing ATP - along microtubules [18-22]. Microtubules are copolymers of α - and β -tubulin that form a dynamic network of filamentous tracks; they extend from a “microtubule organizing center”, where their more stable “minus-ends” are anchored, and point with their fast growing “plus-ends” towards most intracellular destinations [23, 24]. Microtubules have built-in structural polarity that is recognized by the two classes of microtubule motors, the kinesins [25] and the cytoplasmic dyneins [26], and allows them to move in opposite directions along a microtubule. The dyneins move minus-end-directed, while - with few exceptions - the kinesins move plus-end-directed [14]. As a rule – though notable exceptions exist – microtubule motors are multimeric protein complexes containing “motor” subunits – usually two - that bind the microtubules and hydrolyze ATP, and regulatory subunits [27, 28]. Cargo may bind to either the regulatory or the motor subunits [8, 29, 30]. The complexity of the regulatory subunits of dynein by far exceeds that of kinesins [28, 30] (Fig. 1A, B). Subcellular fractionation and immunolocalization studies show that the microtubule motors are mostly present as fully assembled holoproteins, although pools of regulatory subunits – free or present in other protein complexes - are also detected, especially in the case of dynein (see below). Cytoplasmic dynein and its accessory protein complex, dynactin (which is essential for its transport function) form distinct, easily separable entities [31-33].

The majority of microtubule motors carry their cargo by translocating along the microtubules through repetitive steps (caused by conformational changes), and alternative microtubule binding and release of the motor subunits [21, 34-36]. Except for the diffusive movement along microtubules characteristic for the few monomeric kinesins [19], this form of transport is ubiquitous and prevails over other forms of transport, and is therefore considered canonical or “conventional” (Fig. 1).

The cargo typically transported by a microtubule motor in the conventional way is a membrane-bounded vesicular compartment: a trans-Golgi network-derived transport vesicle targeted to the axon terminal or into the dendrites, an endosome moving towards a lysosome, a mitochondrion being transported to a location where ATP is needed. In addition, the microtubule motors transport non-vesicular cargo, such as cytoplasmic protein complexes, including cytoskeletal polymers [37] and RNA-protein complexes [38-40], lipid droplets [41], as well as pathogens that infect cells and hijack the endogenous transport machineries to propagate within cells [42-45]. Microtubule motors also transport large, functionally competent, supramolecular assemblies [46] and particles, such as ribosomes [47, 48]. Regardless of the nature of the cargo, in all these cases, transport is essentially identical to that initially described for the transport of vesicles, with motors attaching to the cargo, and carrying it by “walking” along microtubule tracks. However, motors also generate motility and transport by other mechanisms, as described next.

Unconventional forms of motility

The beating of cilia and flagella

A very different form of motility is powered by the axonemal dyneins [49, 50], which are special dynein motors tethered to the axoneme, a microtubule-based structure that forms the cytoskeleton within cilia and flagella. Dynein-powered movement of axonemal microtubules generates the beating of cilia, and the undulating motion of flagella (see, for review, [51]). Organized in complex inner and outer arm structures, the axonemal dyneins cause sliding of a subset of axonemal microtubules along neighboring microtubules; this sliding is converted into a bending motion of the cilium. The axonemal dyneins were the first microtubule motors to be discovered, and their function has been studied in detail [52] before cytoplasmic dynein was identified as the motor for the conventional, minus-end-directed transport of cargo vesicles along microtubules [53, 54]. Recently, the beating pattern of cilia and flagella in vivo was accurately reproduced in vitro with mixtures of microtubules and microtubule motors, which spontaneously self-assemble into actively bending bundles; at high density, these bundles synchronize their beating in a manner similar to that of dense ciliary fields in vivo [55]. These results suggest that the interactions between molecular motors and microtubules, present above a critical density, are sufficient to spontaneously generate ordered motion that becomes synchronized at multiple levels: within the microtubule bundle, and within a population of densely packed bundles. The mechanisms of synchronization are being investigated.

Microtubule fluctuations

Microtubule motors can move cargo within cells via mechanisms that diverge from the conventional form of transport where motors carry individual cargoes along the microtubules. A case in point is the transport – usually over short distances – caused by longitudinal fluctuations of microtubules with tethered cargo. This form of motility was discovered with simultaneous, high-resolution measurements of displacements of labeled microtubules and peroxisomes in *Drosophila* cells [56]. Such fluctuating rearrangements of entire segments of the cytoskeleton may occur, on small or large scale, in all cells, and could lead to the displacement in unison of both the moving cargo and the stationary organelles attached to the microtubules. The underlying mechanism for these microtubule oscillations includes microtubule sliding over neighboring microtubules, as well as bending of microtubules, often leading to lateral displacement. The oscillations could be caused - at least in part – by the action of cargo vesicles themselves, attaching to two or more microtubules, and crosslinking them, via distinct motor proteins [56] (Fig. 2A). We note that, due to the stochastic nature of these microtubule fluctuations, and the limited possibility for their regulation, it is less likely that they play a role in the targeted transport

of cargo. However, this form of motility may be important for the overall distribution of organelles within localized regions of the cell, especially in the cell body.

Microtubule gliding over long distances

Microtubule gliding could provide a different way of cargo transport over long distances, where carrier vesicles are translocated to their destinations by stably attaching to short, moving microtubules [57]. Our observations on the transport of vesicles in squid axons showed that some vesicles, rather than moving individually along the microtubules, are firmly attached to microtubules that glide over what could be stationary cytoskeletal tracks. Transport of vesicles piggybacking on short microtubules can be reconstituted in motility assays with squid axoplasm, where gliding of vesicle-laden microtubules is frequently observed [57]. The finding that, in axoplasmic spreads, anti-kinesin-1 antibodies heavily decorate microtubule segments, and not attached vesicles ([58], Muresan and Reese, unpublished), is consistent with this unconventional model of vesicle transport, where the microtubules - rather than the associated vesicles - are propelled by the motors. Mechanistically, kinesin-1 could power transport of the short, vesicle-loaded microtubules both anterogradely and retrogradely, within the axon; in each case, the kinesin-1 motors attach to one microtubule with their head domain, and to the other, via their second microtubule-binding site present in the tail domain [59-61]. The two situations differ in whether kinesin-1 binds with its motor domain or with its tail domain to the stationary microtubule (Fig. 2B). In principle, transport in the anterograde direction of the vesicle-laden microtubules could also be powered by cytoplasmic dynein, attached to a stationary actin-spectrin meshwork [57, 62] (Fig. 2B). The extent of this form of transport in the squid axon is not yet established. Importantly, our unpublished data suggest that it might also operate in mammalian neurons [63]. In addition, the results that the disruption of either kinesin-1 or cytoplasmic dynein perturbs vesicular transport in both directions [64] could be explained with this form of transport [57] (Fig. 2B). We note that the transport of short microtubule segments, powered either by kinesin-1 or by cytoplasmic dynein, is reminiscent of the model for delivery of microtubules into axons [65, 66]. Interestingly, a mechanism where cytoskeletal polymers – neurofilaments or short microtubules – serve as carrier structure for nonmembranous protein cargo has been also proposed [37]. Along with the cytoskeletal polymers, a plethora of cytoplasmic proteins, which form the bulk of material transported by what is termed slow axonal transport, could actually move in association with them [37]. Thus, it appears that both soluble cytosolic, and some vesicular, cargo could be transported in association with filamentous, cytoskeletal polymers.

Transport of flexible cargo

Transport of filamentous, flexible cargo, such as the neurofilaments, can also be considered unconventional, in spite of the fact that neurofilaments are carried along microtubules by kinesin-1 or cytoplasmic dynein in a conventional way. As now reported for a variety of cargoes [67, 68], the two motors appear to work in coordination, rather than independently, to transport the neurofilaments either in the anterograde or the retrograde direction [69]. Mechanistically, the coordinated regulation of kinesin-1 and cytoplasmic dynein could be explained either by the reported direct interaction between the intermediate chains of dynein and the kinesin light chains (KLCs) [70], or by corecruitment of kinesin-1 and cytoplasmic dynein to the cargo via adaptor proteins that can interact with both motors, such as JIP-1 [71], JIP-3 [72], or DISC1 [73, 74]. Since neurofilaments are flexible, and could in principle recruit motors along their entire length, they could attach simultaneously – via motors – to more than one microtubule, and be carried along several, adjacent microtubules. If so, the advancement of the motors along the different microtubules could be independently regulated, resulting in movement of different parts of the neurofilament at different rates. However, while neurofilaments appear tortuous in the soma – as predicted by their

flexibility – they appear to be mostly transported as fully extended polymers [69, 75, 76], likely along single microtubules. How this is achieved remains to be established. We note that alternative models for the transport of neurofilaments as subunits rather than polymers have also been proposed [77].

Intraflagellar transport (IFT)

The IFT, representing the movement of cargo within cilia and flagella, defines a form of transport that is both conventional – for soluble protein complexes – and unconventional – for transmembrane proteins. While the transport machinery is most likely the same for both types of cargo, membrane proteins are not transported via vesicles, but by being dragged within the plane of the ciliary membrane (see, for example, [78-81]). The motors that power this motility, kinesin-2 or cytoplasmic dynein 2, “walk” along the axonemal microtubules, while associating with the transported membrane proteins via still incompletely characterized, large protein complexes that include the IFT complexes and the coat forming BBSome, comprised of Bardet-Biedl Syndrome (BBS) proteins [82]. Recently, IFT proteins have been detected outside cilia and flagella, which suggests that IFT-like cargo transport may occur everywhere along the plasma membrane [78]. The seven transmembrane protein, Smoothened (Smo), a protein implicated in the Hedgehog (Hh) signaling pathway, moves from the cell body plasma membrane into the cilia by lateral transport upon Hh stimulation [80]. While the lateral movement of proteins within the plasma membrane is largely regarded as driven by diffusion, the finding that the lateral transport of Smo is regulated by the Hh signal suggests that it may be an active, motor-driven transport. The intricacies of such a transport, whether driven by a myosin motor moving along the subcortical actin filaments, or by a microtubule motor moving along cortical microtubules (Fig. 3) remain to be determined.

Cytoplasmic streaming

A different type of cargo movement – best characterized in *Drosophila* – occurs in the developing oocyte. Termed “cytoplasmic (or ooplasmic) streaming”, it consists of a bulk movement of the ooplasm, which is initially slow, random, and multidirectional, and later becomes a unidirectional, non-saltatory flow [39]. Intended for the mixing and rapid distribution of the cytoplasmic components, including the yolk granules, and for the posterior localization of developmental determinants, such as mRNAs, the cytoplasmic streaming is caused primarily through the action of kinesin-1, and is accompanied by a dramatic rearrangement of the microtubule cytoskeleton [39, 83]. The mechanism by which kinesin-1 generates the cytoplasmic flow is still unclear; one possibility is that it does so by transporting bulky cargoes along microtubules, and stirring in this way the viscous, surrounding cytoplasm [83]. It was also proposed that kinesin-1 could crosslink microtubules, by binding to adjacent microtubules via its head and tail domains, thus causing the movement of microtubules relative to each other [39, 60]. Yet, none of these models can fully explain the complexity of the cytoplasmic streaming, where the requirement for the KLCs is less stringent [39], and the regulatory and ATP-independent microtubule binding functions of the kinesin heavy chain (KHC) tail are questionable [83].

Generation of shape and organelle biogenesis

Generation of shape

The microtubule cytoskeleton, through its rigidity and stability, plays a major role in conferring to cells their shapes. The concerted action of microtubules and microtubule motors is also responsible for the dynamic changes in the shape of plasmalemmal regions, and the generation and maintenance of the fluctuating shapes of organelles. One mechanism by which motors drive changes in shape is microtubule sliding. The beating and bending of

motile cilia and flagella is a typical example of plasmalemmal shape change caused by dynein-powered sliding of axonemal microtubules. Outside cilia and flagella, kinesin-1 appears to be the major motor powering microtubule sliding in interphase cells, by binding to neighboring microtubules via the head and tail domain of KHC [61, 84]. While such sliding normally may not result in cell deformation, plasmalemmal protrusions are generated when the microtubules impinge on the cell membrane [85]. The forces generated by microtubule sliding may drive neurite outgrowth, although extension of neuronal processes might involve additional mechanisms (Fig. 4A).

Organelle biogenesis

The microtubule motors also participate in the formation and generation of tubular organelles, such as the endoplasmic reticulum (ER) [86-89]. In this case, the motors attach to the organelle membrane and cause the formation of membrane tubes by a variety of mechanisms [89, 90]; these have been intensely studied in vitro with reconstituted systems, using purified microsomal membranes [91, 92] or liposomes [93-97]. These studies demonstrated that the motor proteins alone, once bound to the membrane by stable linkages, are capable of generating complex networks of membrane tubes that resemble the ER networks in vivo. The motors that most likely participate in ER biogenesis and maintenance of its structural integrity are kinesin-1 and cytoplasmic dynein [98], the first binding to a still unidentified ER linker protein (possibly, kinectin [99, 100]), and the second binding to acidic phospholipids via organellar spectrin meshworks [29, 95, 101]. Figs. 4B, C illustrate how the membrane bound motors could extract membrane tubes from a reservoir of lipid membrane. The generation and maintenance of the membrane curvature and tubulation in these organelles in vivo also requires specialized proteins capable of bending the lipid bilayer, such as the DP1/REEP/Yop1 and Reticulon families [102-104]. While ER formation is a complex process that relies on the concerted action of many different molecular machines, the activity of microtubule motors, and the integrity of the microtubule cytoskeleton, are essential.

The deformation of, and extraction of processes from, the membranes of organelles by the action of the microtubule motors constitutes the basis of shape generation of a variety of organelles, including the endosomes and the Golgi apparatus, whose structural integrity is microtubule- and motor- dependent. Finally, the microtubule motors participate, along with other specialized, coat-forming, and small GTP-binding proteins, in vesicle budding and membrane tubulation, including the generation of the early endocytic vesicles [105], or of the tubes of ER-to-Golgi intermediate compartment (ERGIC) [95, 106]. Of course, these activities rely on the presence of microtubules at the sites of membrane invagination.

Static anchors and structural components

Aside from their role in motile events, microtubule motors function as anchors for the transported cargo at their destination and at certain points during transit. The transport along microtubules is often discontinuous, with pauses occurring when the motor stops, while the cargo remains anchored – presumably via the motor – to the microtubule. This behavior is exacerbated for large organelles, such as mitochondria and peroxisomes, which spend most of the time being stationary and attached to microtubules [107]. Together with the motors, organelle-bound microtubule-associated proteins (MAPs), or other bona fide anchor proteins [108], could participate in this anchoring. Cytoplasmic dynein anchors the astral microtubules to the cell cortex, a process that is thought to participate in spindle positioning during mitosis [109]. While in this case dynein still “walks” on the microtubules while they slide along the plasma membrane [109, 110], the result is that of anchoring of the microtubules to the plasmalemma. Cytoplasmic dynein also “anchors” the Golgi apparatus at its characteristic pericentrosomal location [111]; this process also requires active, dynein-

powered motility along the microtubules emanating from the microtubule organizing center. Yet, in other cases, the microtubule motors – both kinesins and cytoplasmic dynein – serve as bona fide, static anchors for mRNA-protein complexes or vesicular compartments, most likely without the implication of their motor activity. For instance, in the *Drosophila* blastoderm embryo, cytoplasmic dynein becomes a static anchor for several mRNAs that attain apical localization after transport [112]. Importantly, this anchoring is independent of the ATPase activity of dynein. Also, in *Drosophila* oocytes, cytoplasmic dynein becomes an anchor at the destination for the *gurken* mRNA cargo [113]. In this case, the mRNA cargo is not anchored to the microtubule tracks, but to the sponge bodies, which are electron-dense, cytoplasmic structures of ill-defined function. While the transport of mRNA requires the entire dynein-dynactin complex, its anchoring to the sponge bodies relies on dynein alone. Surprisingly, dynein is an integral part of the sponge bodies, and its function, but not the presence of microtubules, is required to preserve the structural integrity of the sponge bodies [113]. A strikingly similar role appears to be played by a kinesin-2 motor, KIF3, in the photoreceptor cells of vertebrate retina. While KIF3 is one of the motors that participate in trafficking of cargo into the outer segments of photoreceptors [114-116], the KIF3A subunit of this kinesin is abundantly present at the ribbon synapses [117], a specialized form of synapse established by photoreceptor and bipolar cells in the retina, by pinealocytes, and by hair cells [118]. The function of kinesin-2 at the synapse, while essential [119], remains unclear. The association of KIF3A with the synaptic vesicles docked at the synaptic ribbon – an amorphous structure with likely role in synaptic vesicle release – suggests that KIF3 transports the precursors of synaptic vesicles into the photoreceptor axon. Intriguingly, KIF3A is also an integral part of the ribbon matrix [117], which is reminiscent of cytoplasmic dynein as part of the sponge bodies in *Drosophila* oocytes [113]. Thus, it is likely that, at the synapse, this kinesin-2 switches from a microtubule motor to a static anchor for the transported cargo, and to a structural component of the ribbon, a hypothesis that remains to be tested. Another kinesin motor, kinesin-1, was shown to localize at the ciliary rootlet of retinal photoreceptor cells [120]; the ciliary rootlet is a large, fibrous structure that emanates from the basal body and extends towards the synapse [121]. The KLCs of kinesin-1 interact with rootletin, a major component of the ciliary rootlet [120]. Since the bona fide cargo protein of kinesin-1, the amyloid- β precursor protein (APP) [122-124], also localizes at the ciliary rootlet, it was proposed that kinesin-1 could provide a static anchor for some kinesin-1 cargo proteins at the rootlet [120]. Anchoring cargo at the destination, or at intermediary stations along the itinerary, appears to be a widespread function of the microtubule motors.

Role in cell signaling

For over a decade, the microtubule motors have been considered major players in the regulation of cell signaling. However, in most cases, the role of the motors in signaling was viewed solely as carriers of signaling molecules – sometimes assembled into complexes [125, 126]. For example, the kinesin-2 motor, KIF17, transports into dendrites the NMDA receptor complex, assembled on the molecular adaptor, mLin-10 [127], and regulates its subunit composition [128]. Similarly, kinesin-1 transports the c-Jun N-terminal kinase (JNK) signaling complex into neurites; this complex includes the upstream kinases, assembled on one of the adaptors, JNK-interacting protein-1/2/3 (JIP-1/2/3) [129]. Another example is that of microtubule motors that participate in the formation of primary cilia, and that transport cargo – including signaling molecules – into and out of the cilia [130]; in mammals, this role is accomplished by the kinesin-2 motors, KIF3 [131, 132] and KIF17 [133], the kinesin-4 motor, KIF7 [134], and the two cytoplasmic dynein complexes – conventional and ciliary [135-138]. This long-neglected organelle is an organizing center for many signaling pathways in mammalian cells, most notably the Wnt and Sonic Hedgehog (Shh) signaling [139]. Importantly, the primary cilia are not only the sites where some

signaling complexes are concentrated, but they actively participate, via a motor-driven rotational movement, in the generation of the extracellular fluid flow that distributes secreted signaling molecules – in a concentration gradient – to distantly located target regions, as occurs during embryonic development [131, 132, 140]. Yet, the notion that “kinesin carries the signal”, used to summarize the role of kinesins in transporting kinase complexes to their site of action, turned out to define only one facet of the involvement of microtubule motors in signaling.

Functions within the nucleus: regulation of transcription

Several studies have shown that the microtubule motors regulate transcription – both in neuronal and non-neuronal cells – with important consequences for the cell physiology. For example, the kinesin-4 motor, KIF4A, regulates the neuronal survival by suppressing the enzymatic activity of poly (ADP-ribose) polymerase-1 (PARP-1) [141], a DNA-repairing enzyme and transcriptional modulator, which acts by altering chromatin structure [142]. The testis-specific kinesin-2 motor, KIF17B, regulates the cAMP-response element modulator (CREM)-dependent transcription, which is essential for spermatogenesis, by controlling the localization, and thus the activity, of the transcriptional coactivator, activator of CREM in testis (ACT) [143]. In both cases, the kinesin motor directly interacts with the transcription factor, PARP-1 or ACT, and suppresses their activity by physically blocking their functional interaction with partner proteins (Fig. 5A). Interestingly, the motor activity of these molecular motors is not required for this regulation of transcription; headless truncation mutants of both motors are fully active as transcriptional regulators [141, 144]. The interaction of KIF4A and KIF17B with the transcription factors is regulated by the phosphorylation of either the motor protein, or the interacting factor [141, 144]. As expected, the function of these motors as transcriptional regulators requires their translocation into the nucleus. Indeed, both motors shuttle between the cytoplasm – where they mostly reside – and the nucleus, by employing the machineries for nuclear import and export [141, 144]. Other kinesins may also have functions within the nucleus; for example, the kinesin-2 motor, KIF17, shuttles between the cytoplasm, primary cilium, and nucleus in a regulated manner [145].

Functions outside the nucleus

Clear roles in signaling were identified for two atypical kinesins, KIF26A [146], a kinesin-11, and the *Drosophila* kinesin-4 member, Costal2 [147]. Phenotypically, mice deficient in KIF26A show anomalies in the development of the enteric nervous system, a site of action of KIF26A. This is a result of hyperactivation of glial cell-derived neurotrophic factor (GDNF)-RET signaling, caused by uninhibited formation of downstream signaling complexes mediated by the adaptor protein, Grb2. Mechanistically, KIF26A negatively regulates the formation of these signaling complexes, by directly binding, via its tail domain, to Grb2, and blocking its interaction with its binding proteins [146]. Thus, similar to KIF4A and KIF17B, the KIF26A kinesin interferes with the formation, and therefore activation, of signaling complexes (Fig. 5B). Interestingly, KIF26A, while preserving the typical structure of a kinesin motor, with head and tail, as well as the capability to bind to microtubules, does not possess ATPase activity, and therefore cannot transport cargo [146]. Thus, its primary function could be to regulate the GDNF-RET signaling. Another unconventional kinesin, the *Drosophila* Costal2, plays multiple roles in Hh signaling [148, 149], a pathway mostly active during embryonic development that also regulates tissue homeostasis in adults [150]. At least one of these roles does not rely on transport, but rather on the ability of Costal2 to function as platform for recruitment of a Hh-specific kinase complex to its substrate, the transcription factor Cubitus interruptus (Ci); this allows the phosphorylation of Ci, and its conversion, via proteolysis, into a transcriptional repressor [147]. Thus, the kinesin, Costal2 is a key player in Hh signaling, by directly

regulating the phosphorylation of Ci, a function independent of transport (Fig. 5C). In fact, Costal2 was initially believed to lack motor activity. Although more recent reports indicate that it may possess motile properties [151], its *in vivo* function as a motor is still questionable. Surprisingly, kinesin-1, by far the most abundant motor for anterograde transport in most cells, appears to participate in the phosphorylation of cargo proteins to which it binds. Recent reports suggest that it might do so by acting – not as a motor – but as scaffolding protein that recruits active kinase complexes to vesicle-associated kinesin-1 linkers [152, 153]. A case in point is the kinesin-1-dependent phosphorylation by JNK of APP [152, 154], a protein relevant to Alzheimer's disease that is transported by kinesin-1 within neurons [124, 155]; this phosphorylation event is initiated by the recruitment of kinesin-1 to APP at the endoplasmic reticulum [156]. Mechanistically, kinesin-1, via its two light chains, provides the link between the JNK complex – assembled on the scaffolding protein, JIP-3 – and APP, via the adaptor protein, Fe65, thus allowing for APP phosphorylation (Fig. 5D). It was proposed that this phosphorylation is part of a signaling cascade triggered in neurons by the overaccumulation of kinesin-1 in the cell soma, when axonal transport is impeded. The above described phosphorylation of APP at the ER, facilitated by kinesin-1, could be part of a signaling pathway aimed at correcting the axonal transport deficiency [156]. Although the kinesin-1-dependent phosphorylation might preferentially target cargo proteins destined for transport, as well as kinesin-1 itself [157], we note that it does not require the motor activity of kinesin-1, and could be independent of transport. This function of kinesin-1 relies entirely on its ability to provide a platform – via the two KLCs – for assembling kinase complexes in the vicinity of a cargo-associated substrate. Thus, kinesins participate in cell signaling in multiple ways: by transporting signaling complexes to their site of activity, by regulating the interactions between proteins that participate in signaling cascades, and by enabling the phosphorylation of target proteins by co-recruited kinase complexes.

Motor-independent signaling functions of subunits of microtubule motors

Although integral part of molecular motors, non-motor subunits of kinesins and cytoplasmic dynein have also been detected outside motors, apparently not associated with the corresponding motor subunits. For instance, the dynein light chains, DYNLL (LC8) and DYNLT3 (rp3), localize in part to the nucleus and possess regulatory functions for transcription. Thus, LC8 participates in the induction of the estrogen-receptor-regulated genes, by directly interacting with the estrogen receptor, as well as with the regulatory elements of the estrogen receptor target genes [158]. Some reports suggested that these nucleus-localized light chains are part of the dynein motor complex, and possibly regulate the transcription by motor-dependent rearrangement of the nuclear components [159], or by assisting transport of signaling proteins into the nucleus (a chaperone-like function) [158]. Another report showed that the regulation of transcription by the dynein light chain, rp3 could occur in a dynein-independent fashion [160], possibly by physically blocking the interaction between the components of the signaling complexes. The non-motor subunits of microtubule motors, likely acting independent of the motor, also regulate signaling events within the cytoplasm. For example, the KAP3 polypeptide, normally part of the KIF3 kinesin-2 motor, is a potential regulator of small G proteins, functioning in association with a GDP dissociation stimulator [161]. Also, a dynein light chain, LC8, interferes with the interaction of p38, a mitogen-activated protein kinase, with the upstream kinase, MKK3/6, and prevents the phosphorylation – and thus activation – of p38 [162]. This is mechanistically similar to how the kinesins KIF4A, KIF17B, and KIF26A interfere with signaling (see above). Interestingly, the inhibition of the p38 pathway by LC8 is at the core of the mechanism by which cAMP blocks p38 activation, leading to the suppression of NF- κ B signaling and to apoptosis. LC8 appears to engage in a variety of other dynein-independent interactions with signaling proteins, such as the neuronal nitric oxide synthase,

I κ B α , the proapoptotic factors Bim and Bmf, in a highly regulated fashion (see for review [163], and below). Finally, the *C. elegans* dynein light chain, DYRB-1, a member of the DYNLRB/roadblock/LC7 family, is part of the heteromeric G protein signaling pathway that controls spindle positioning, likely via the astral microtubules [164].

The remodeling of the cytoskeleton in interphase and mitosis

The microtubule motors participate in the remodeling of the cytoskeleton, acting primarily on microtubules, but also on the actin network. The motors cause changes in the distribution of microtubules relative to each other in the network, or modifications of single – or groups of – microtubules. The first type of changes comprises the crosslinking of microtubules, and the sliding of microtubules relative each other, or against an actin meshwork. In the second category, we include the regulation of the microtubule dynamics – disassembly, elongation, and stability – and of the posttranslational, chemical modifications that target tubulin within the microtubule polymer.

Major cytoskeletal remodeling events occur both during interphase and mitosis; the most dramatic changes occur at mitosis, when the network of interphase microtubules collapses and is replaced by the mitotic spindle – a microtubule-based structure – in a process that is reversed after cytokinesis. Multiple microtubule motors are active during mitosis – most being localized to the mitotic spindle – and work coordinately during spindle assembly, chromosome congression, separation of chromatids, spindle elongation and dissolution. While cytoplasmic dynein is an important part of the mitotic machinery, kinesin-1, the major microtubule motor for intracellular transport in interphase, is conspicuously “silent” during this stage, with the “mitotic” kinesins becoming active. Despite of their complexity, most functions of the mitotic motors are cytoskeletal remodeling activities: microtubule sliding against each other, and microtubule elongation and shortening, achieved by polymerization and depolymerization. Some of these activities are briefly discussed below; the mechanistic details of the microtubule remodeling processes during mitosis are excellently covered elsewhere (see, for example, [109, 165-172]).

Regulation of microtubule dynamics

The microtubules are dynamic structures that undergo alternate cycles of elongation and shortening, a behavior described as dynamic instability; the transitions from elongation to shortening are called catastrophes, and those from shortening to elongation, rescues [173-176]. This dynamics is determined by events that occur at their ends, primarily the plus-ends. Within cells, the microtubule dynamics is largely regulated by the plus-end tracking proteins (+TIPs) that bind to, and accumulate at, the fast growing plus-ends of the microtubules, and regulate the rates of polymerization and depolymerization [177, 178]. The kinesins that participate in the regulation of the microtubule dynamics also act primarily – although not exclusively – at the plus-ends, and they do so in several ways. First, the kinesins are responsible for the transport of +TIPs to the plus-ends of the microtubules; this conventional function is carried out, for example, by kinesin-1, transporting CRMP2 [179], and the kinesin-2 motor, KIF17, transporting APC [180] to the plus-ends of microtubules.

Microtubule disassembly—In a less conventional way, kinesins act as microtubule depolymerases, by promoting their disassembly and shrinking, or tubulin polymerases, promoting microtubule elongation. The depolymerizing kinesins belong to the kinesin-5, kinesin-8, kinesin-13, and the minus-end-directed, kinesin-14 families. Some of them initiate the depolymerization by facilitating catastrophes (e.g., the kinesin-13, KLP10A), while other suppress rescue of microtubules, and thus entrain depolymerization (e.g., KLP59C) [181-183]. Interestingly, the mitotic motor, MCAK, another kinesin-13, which slides on microtubules in both directions by diffusion, disassembles both microtubule ends

[166, 184]. The kinesin-8 motors show the remarkable property of depolymerizing the longer microtubules faster than the shorter ones, acting always at the plus ends [185, 186], a function that is conserved from yeast to humans. In this ways, these motors control the length of microtubules, an important task both during mitosis and interphase. Interestingly, while many of the depolymerizing kinesins act as catastrophe factors, they control the microtubule depolymerization in distinct ways [187]. Unlike the kinesins, the cytoplasmic dynein motor may not have an important role in regulating the dynamics of microtubule plus-ends; yet, it accumulates at the plus-ends, either through transport by kinesins, as cargo, or by being directly recruited to +TIPs [178]. Noteworthy, p150^{Glued}, a subunit of dynactin, possesses +TIP activity [188].

Microtubule elongation—While numerous kinesins are microtubule depolymerases, only a few have been shown so far to participate in microtubule elongation, and the mechanisms involved are poorly understood. One of the better-characterized examples of this group is the kinesin-7, CENP-E, a mitotic kinesin that associates with the kinetochore and functions in chromosomes congression [189, 190]. The model for CENP-E-promoted microtubule elongation envisions that the addition of the $\alpha\beta$ -tubulin dimers to the microtubule end is tightly coupled to ATP turnover [191]. Other kinesins with microtubule elongation activity are the yeast Kip2p motor [192], and the *Drosophila* Nod, a pseudo-motor with no motile function that promotes microtubule polymerization in vitro [193]. Unexpectedly, kinesin-1 also appears to control microtubule elongation, yet in an indirect way, by functioning within a regulatory signaling event. Indeed, the concerted action of kinesin-1 and JNK was recently proposed to promote microtubule elongation, most likely by enabling the phosphorylation, by kinesin-1-bound JNK, of unidentified proteins at the microtubule tip [194, 195]. Although this process likely occurs concomitantly with the elongation of microtubules and the movement of kinesin-1 along the microtubule, the phosphorylation of the tip associated proteins – if it does occur – is likely independent of the motor activity of kinesin-1. While cytoplasmic dynein itself is not known to play a role in microtubule elongation, the dynactin component, p150^{Glued} was recently shown to catalyze nucleation, and to promote polymerization of, microtubules [196].

Microtubule stabilization—The JNK-mediated effects of kinesin-1 on microtubule dynamics discussed above also include stabilization, achieved by mechanisms that include the stimulation of microtubule rescue events [194]. The yeast kinesin, Tea2, and the mammalian kinesin-2 motor, KIF17 also stabilize microtubules, by accumulating at their plus ends, and by participating in the formation of a complex, which includes +TIPs, that caps the microtubule plus-ends to attenuate polymerization/depolymerization rates [180, 197]. Thus, kinesin-1 and KIF17 may promote microtubule stabilization both directly, as part of the microtubule cap complex, and indirectly, as transporters of capping proteins (see above). The kinesin-4 motor, Xklp1 (chromokinesin), strongly inhibits the microtubule dynamic instability – both the shrinkage and the growth – in vitro, possibly by causing structural changes in the microtubule lattice [198]. In addition, kinesins could promote the stabilization of microtubules by firmly binding to them, and acting as MAPs. In support of this mechanism, a recent study showed that kinesin-1 binds, via its tail domain, to a site that overlaps with the binding site of Tau (a MAP), causing assembly and stability of microtubules in a manner similar to Tau [60].

Role in microtubule network organization: microtubule sliding and crosslinking

The organization of the microtubule cytoskeleton is determined not only by the microtubule dynamics, but also by their repositioning relative to each other and within the network. These rearrangements are achieved by the sliding and bending of microtubules, and by the dragging of the microtubule tips along more robustly anchored microtubules. These events

require the action of motors. The existence of microtubule binding sites both in the head and tail of kinesin-1 allows this motor to mediate microtubule crosslinking. As a consequence, kinesin-1 plays an active role in the organization of the microtubule cytoskeleton, by bridging and sliding the microtubules. Indeed, studies done in the fungus *Ustilago maydis* showed that Kin1, a kinesin-1, participates in microtubule bundling and bending in vivo [61]. Other kinesins, and also cytoplasmic dynein, enact microtubule gliding, mostly but not exclusively during mitosis, controlling the length of the mitotic spindle during the mitotic stages [168]. The members of the kinesin-5 family, e.g., Eg5, have a bipolar, tetrameric structure, which allows them to crosslink and slide antiparallel microtubules in the mitotic spindle [167, 169]. Kar3, a yeast kinesin-14, generates in vivo at least two distinct motors by dimerizing with the non-motor proteins Cik1 and Vik1 [199]. Although neither Cik1 nor Vik1 bind nucleotides, they are capable of interacting – at least transitory – with microtubules, which confers to Kar3Cik1 and Kar3Vik1 motors microtubule crosslinking properties [200]. Notably, Kar3Cik1 is also a microtubule depolymerase, acting at the microtubule plus-ends [201].

Remodeling of the actin cytoskeleton

Evidence is emerging that the microtubule motors, or their subunits, also participate in the remodeling of the actin cytoskeleton. They certainly do so in a conventional way, by transporting regulatory proteins to the sites where active actin polymerization/depolymerization occurs, such as growth cones. Less conventional is the capability of members of the kinesin-6 and kinesin-12 families to directly interact with actin filaments, an interaction that is required, for example, during cytokinesis, or axon navigation and branching [202-204], processes that rely heavily on the actin cytoskeleton. Also, members of the kinesin-11 family – the mammalian KIF26B [205] and the yeast Smy1p [206] – have been shown to physically interact with, and modulate the activity of, nonmuscle myosins, and thus indirectly regulate tension in actin filaments. In another unconventional way, motor subunits regulate, or are part of, the signaling pathways that control the actin dynamics. Such might be the role of Tctex-1 (DYNLT1), a cytoplasmic dynein light chain [207] normally implicated in targeting and cargo recruitment [208]. During the outgrowth of neurites, Tctex-1 has a dynein-independent role in actin remodeling, likely acting through the Rho GTPases that control the actin cytoskeleton dynamics [209]. As reported, Tctex-1 can cause both actin destabilization and polymerization during the various steps of growth cone dynamics.

A plethora of other unconventional functions

Microtubule motors have been implicated in numerous cellular processes (other than those described above), where they do not employ their cargo transport activity. They participate in cytokinesis, primarily by regulating microtubule dynamics (see, for example, [210]). The mitotic motor, kinesin-5, which functions in microtubule crosslinking and sliding, has also roles in postmitotic neurons, where it regulates axon growth [211]. Curiously, instead of propelling movement, it appears to restrict the transport of the short microtubules that are exported into axons during growth. It does so, by acting as a break – both active (hydrolyzing ATP) and passive – on the transport performed by other motors, opposing microtubule transport preferentially in the anterograde, but also in the retrograde, direction [211]. Notably, kinesin-5 also antagonizes various microtubule motors during mitosis [212, 213]. In many cases, there is clear evidence that microtubule motors are implicated in unexpected cellular functions, although their specific roles and mechanisms of action are not fully understood. So is the function of the kinesin-3 motor, KIF1B β , which participates in the regulation of apoptosis, and acts as tumor suppressor relevant to certain tumors of neural origin, a role that does not require its motor domain [214], but mechanistically remains unexplained. Also, a fraction of the kinesin-2 motor, KIF3, appears to reside within the

nucleus, where it associates – via the KAP3 nonmotor subunit – with the human chromosome-associated polypeptide HCAP, a protein required for chromosome dynamics throughout the cell cycle [215]. Surprisingly, microtubule motors are required during translation, as is the case of the kinesin-5 motor, Eg5 [47]. Eg5 also functions in the transport of ribosomes. However, while motors are known to transport and localize mRNA-protein complexes to sites of translation [216], their participation in enhancing translation – as reported for Eg5 – may require not only their motile function, i.e., promotion of ribosomal transit, but also roles as elongation or termination factors [47]. Interestingly, a kinesin-3 motor, KIF1A, appears to play an opposite role during translation of some transcripts implicated in autism spectrum disorders: instead of moving ribosomes, this motor appears to participate in stalling the ribosomes on their target mRNAs [217].

Non-motor subunits of molecular motors are broad specificity adaptors for protein-protein interactions

The light chains of cytoplasmic dynein, Tctex-1 and LC8, already mentioned in previous sections of this review, deserve special attention. While they are part of the cytoplasmic dynein motor complex, they are also detected at significant levels as free polypeptides, or as part of protein complexes unrelated to dynein. In this way, they regulate a large variety of cellular functions – some still ill defined – such as the role of Tctex-1 in neural progenitors in the adult brain, where this dynein component alone is present at unusually high levels [218]. The dynein light chain LC8 also functions in redox state sensing, allowing for redox-dependent regulation of activation/inhibition of the transcription factor, NF- κ B [219]. It also forms multimeric complexes with myosin V [220], neuronal nitric oxide synthase [221], or Swallow (a protein involved in mRNA localization in *Drosophila*) [222], and with several apoptotic factors (see for review [163]). LC8 functions mostly as a dimer, whose assembly is controlled by phosphorylation and pH [223, 224]. Notably, it is the LC8 dimer that becomes incorporated into the various protein complexes. Recent studies show that LC8 plays an active role in the formation of these complexes, acting as a “dimerization hub”, i.e., a facilitator of dimerization for other proteins in the complex [225, 226]. Non-motor subunits of kinesins also contain motives known to participate in protein-protein interactions, such as the tetratricopeptide repeat (TPR) domains of the KLC of kinesin-1 [227] and the Armadillo repeats of the accessory subunit, KAP3, of the mammalian kinesin-2 motor, KIF3, and of its homologues in other species [228]. It is tempting to assume that many of the interactions established by the non-motor subunits with proteins that do not fit to our existing knowledge are “non-specific”. Yet, in most cases such unexpected interactions have proven to be biologically significant, unraveling novel, unconventional functions of the motors. Generalizing, it appears that the non-motor subunits of molecular motors are adaptors with broad specificity that participate in a plethora of complexes with function in cell signaling, in addition to their role in motor regulation and cargo recruitment. The motor subunits could also have roles independent of the non-motor polypeptides, although these aspects are largely unexplored.

Conclusions

By fulfilling their “conventional” transport function, the microtubule motors have a profound regulatory role on most intracellular processes, encompassing everything from common housekeeping tasks to highly specialized signaling events. However, as discussed above, occasionally, even the transport function deviates from the ordinary, involving novel forms of translocating the cargo, or carrying atypical cargo. Remarkably, the microtubule motors also perform numerous tasks that do not necessarily involve transport; some even do not require their motor activity. Often, non-motor subunits may act independently of the motor subunits, either alone or as part of other protein complexes, which may have little in

common with the motor. Yet, these unconventional functions are the ones that will bring future surprises, and open up the field of molecular motors towards new directions. Certainly, these unconventional functions are equally important for the physiology and pathology of the cell as their transport function.

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Highlights

- Microtubule motors have numerous functions that are not related to their main function in cargo transport
- Some functions of microtubule motors do not require their motor activity
- Microtubule motors modulate interactions between signaling proteins, including transcription factors
- Microtubule motors facilitate phosphorylation by recruiting kinases to their substrates
- Microtubule motors generate movement of cellular constituents by a variety of unconventional mechanisms

Figure 1.

Conventional transport of cargo by kinesins and cytoplasmic dynein. (A, B) Diagram of the structure of kinesin-1 (A) and cytoplasmic dynein-dynactin complex (B). Kinesin-1 is shown in the inhibited conformation, i.e., not bound to the cargo and microtubules, which is caused by an interaction between one tail domain of the heavy chain (KHC) and the two motor domains [229, 230]. An interaction between the light chain (KLC; red) and the motor domain has also been reported [231]. Cytoplasmic dynein consists of: two heavy chains (DHCs) that contain the motor domains and the microtubule binding domains (which extend away from the motor domain); light intermediate chains (LICs), intermediate chains (ICs), and several light chains (Roadblock, LC8, Tctex1) [26]. Only two subunits of the dynein accessory complex, dynactin – p150^{Glued}, containing microtubule binding sites, and the short filament formed by polymerized ARP1, usually involved in cargo binding – are shown. Boxed components of dynein-dynactin are depicted as a black box in (C, D). The dynein regulatory protein complex formed by Lis1, Ndel1, and 14-3-3 ϵ [232] is not drawn. Note that the subunits are not necessarily drawn to scale, but show their relative position in the molecule. Also, the dynein motor domain is ~3X the size of the kinesin-1 motor domain. (C) Small scale representation of kinesin-1 and dynein-dynactin complex. (D) Directional transport of vesicles along microtubules by recruiting kinesin-1, cytoplasmic dynein, or both motors via membrane-associated, linker proteins or protein complexes (the linkers are not drawn). When both motors are associated with the cargo vesicle, direction of movement towards microtubule plus- or minus-ends is determined by coordinated regulation of both motors, which may interact directly [70], or via adaptor proteins that can simultaneously bind both motors [71-74].

Figure 2.

Unconventional forms of microtubule motor-driven movement (A) and transport (B) of membrane bounded cargo. (A) Oscillations of microtubules, powered by the interaction of cargo-bound motors of opposed polarity with adjacent microtubules [56], move the cargo over short distances, perpendicular (as drawn) or parallel (not shown) to the longitudinal axis of the microtubule bundle (see [56]). As depicted here, microtubule movement is generated through the simultaneous interaction with two microtubules of mitochondria, which bind both kinesin-1 and cytoplasmic dynein. (B) A model for anterograde and retrograde transport of cargo vesicles by kinesin-1-driven gliding of short microtubules over long, stationary microtubules. Vesicles are firmly attached to the moving microtubule via anchoring proteins (not drawn). For anterograde transport, the vesicle-laden, short microtubule is itself a cargo for kinesin-1, attached to kinesin-1's tail (a). For retrograde transport, kinesin-1 motors are anchored (via their tail) to stationary microtubule tracks, and the vesicle-laden, short microtubule is pushed in the retrograde direction (b). This mechanism could explain why function-blocking, anti-kinesin-1 antibodies inhibit vesicle transport in both directions, since both plus-end- and minus-end-directed transport is powered by kinesin-1. An alternative model, where short microtubules are transported anterogradely through the action of cytoplasmic dynein, anchored to a stationary actin-spectrin meshwork, was proposed to explain axonal transport of short microtubules [66, 233]; in a similar way, cytoplasmic dynein could power transport of vesicle-laden microtubules (c). Additional scenarios for anterograde and retrograde cargo transport in association with moving microtubules can be envisioned.

Figure 3.

Motor-driven, lateral transport of proteins within the plasma membrane. The model presented in this diagram proposes that some membrane proteins could be dragged laterally, within the plane of the membrane, by associating with molecular motors that move along subcortical microtubules, in regions where the subcortical actin cytoskeleton is permissive for such movement. This form of motility is similar to the transport of membrane proteins within cilia and flagella, which associate with IFT particles for movement along the ciliary/flagellar membrane.

Figure 4.

Motor-driven generation of shape and organellogenesis. (A) Generation of protrusions and cellular processes – including neurites - by additive sliding of microtubules over neighbouring microtubules. This process is powered by kinesin-1, which attaches to adjacent microtubules via its tail and motor domains (see [85]). (B, C) In vitro shaping of complex, ER-like networks of membrane tubes from a large membrane reservoir to which microtubule motor proteins bind. (B) The video-enhanced differential interference contrast image shows the membrane tube network generated from liposomes and purified dynein-dynactin and spectrin. Dynein-dynactin binds to the spectrin meshwork that associates, via the pleckstrin homology domain of β -spectrin, with the acidic phospholipids contained in the liposomes [29]. The lipid reservoir consists of a cluster of liposomes. The diagram in (C) depicts the two mechanisms for tube extension from a large liposome cluster: (1) by extension along a microtubule; and (2) by attaching of a generated tube to another microtubule, via its tip, followed by dragging of the tip along the microtubule. Formation of a three-way junction, typical for the ER networks, is shown in the bottom left part of the image.

Figure 5.

Unconventional roles of kinesins in cell signaling. (A, B) Kinesins interfere with the normal interaction between nuclear (A) or cytoplasmic (B) signaling molecules. (A) The kinesin-4, KIF4, binds to the nuclear protein, poly (ADP-ribose) polymerase-1 (PARP-1), a DNA-repairing enzyme and transcriptional modulator, and prevents its interaction with chromatin remodeling proteins [141]. Phosphorylation of PARP-1 by calcium-calmodulin kinase II (CaMKII) leads to the release of KIF4, thus allowing its interaction with, and inactivation of, the chromatin remodeling proteins. (B) The atypical kinesin-11, KIF26A, blocks glial cell-derived neurotrophic factor (GDNF)-RET signaling by interfering with the interaction between the downstream adaptor proteins, SHC and GRB2, thus preventing propagation of the signal, and subsequent activation of the phosphatidylinositol-3 kinase (PI3K) and mitogen activated protein kinase (MAPK) signaling pathways [146]. The receptor tyrosine kinase, RET, is shown in the active, phosphorylated form (pRET). GFR α 1 is a glycosyl-phosphatidylinositol-anchored coreceptor. (C) Costal2 (Cos2), a *Drosophila* kinesin-like protein with disrupted ATPase activity participates in Hedgehog (Hh) signaling. Cos2 acts as scaffolding protein for recruiting a complex of three kinases – protein kinase A (PKA), casein kinase I (CKI), and glycogen synthase kinase 3 (GSK3) – to their substrate, the transcription factor, Cubitus interruptus (Ci). In the absence of Hh signal, the transmembrane protein, Smoothed (Smo) is “repressed”, and the three kinases phosphorylate Ci, priming it for proteolysis. The generated Ci fragment, Ci⁷⁵, then translocates to the nucleus and acts as repressor of target genes. Activation of the Hh pathway “de-represses” Smo, leading to the release of the kinase complex, followed by phosphorylation of Cos2 through activation of the Ser/Thr kinase, Fused (Fu). As a consequence, the full-length, transcriptionally active Ci¹⁵⁵ is released from Cos2, escaping proteolysis, and translocates to the nucleus to trigger expression of target genes. (D) Kinesin-1 binds – through one of its KLCs - to the amyloid- β precursor protein (APP), via the adaptor protein, Fe65, and recruits, via its second KLC, an active c-Jun N-terminal

kinase (JNK) complex, assembled on the JNK interacting protein-3 (JIP-3). This leads to phosphorylation of Thr⁶⁶⁸ of the ER-localized APP, followed by its amyloidogenic cleavage. The phosphorylation of APP could be triggered by the accumulation of kinesin-1 in the cell body of neurons with disrupted axonal transport. Details on the signaling pathways included in this figure are given elsewhere [142, 156, 234, 235].