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Review

Kinesin superfamily proteins and their various functions and dynamics

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Abstract

Kinesin superfamily proteins (KIFs) are motor proteins that transport membranous organelles and macromolecules fundamental for cellular functions along microtubules. Their roles in transport in axons and dendrites have been studied extensively, but KIFs are also used in intracellular transport in general. Recent findings have revealed that in many cases, the specific interaction of cargoes and motors is mediated via adaptor/scaffolding proteins. Cargoes are sorted to precise destinations, such as axons or dendrites. KIFs also participate in polarized transport in epithelial cells as shown in the apical transport of annexin XIIIb-containing vesicles by KIFC3. KIFs play important roles in higher order neuronal activity; transgenic mice overexpressing KIF17, which transports *N*-methyl-D-asp (NMDA) receptors to dendrites, show enhanced memory and learning. KIFs also play significant roles in neuronal development and brain wiring: KIF2A suppresses elongation of axon collaterals by its unique microtubule-depolymerizing activity. X-ray crystallography has revealed the structural uniqueness of KIF2 underlying the microtubule-depolymerizing activity. In addition, single molecule biophysics and optical trapping have shown that the motility of monomeric KIF1A is caused by biased Brownian movement, and X-ray crystallography has shown how the conformational changes occur for KIF1A to move during ATP hydrolysis. These multiple approaches in analyzing KIF functions will illuminate many basic mechanisms underlying intracellular events and will be a very promising and fruitful area for future studies.
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Introduction

The neuron is composed of a cell body, dendrites, and a long axon. In the axon, most of the required proteins must be transported from the cell body. In the dendrite, some selected species of mRNA are transported and translated locally, but again, the majority of proteins are transported from the cell body. Both in the axons and dendrites, microtubules run longitudinally and function as rails for transport. Kinesin superfamily proteins (KIFs) and cytoplasmic dyneins serve as motors that move along microtubules carrying cargoes such as membranous organelles, protein complexes, and mRNAs [1–3]. Anterograde axonal transport (i.e., transport in the direction from the cell body to the synaptic terminal) is carried out by KIFs. Anterograde dendritic transport is also carried out by KIFs. Some molecules are retrogradely (from neurite terminals to the cell body) transported and these transports are carried out mostly by cytoplasmic dynein. The components of dendrites and axons differ considerably, and therefore proteins need to be properly sorted and selectively transported.

Axonal and dendritic transports serve as ideal systems for studying motors involved in intracellular transport. However, studies have also shown that transport mechanisms found in neurons are operational in other cell types as well. Therefore, the principles revealed in axonal and dendritic transports are likely to serve as foundations for elucidating the mechanisms of intracellular transport in general.

After briefly summarizing the basic knowledge on microtubules and KIFs, we will focus primarily on the most recent findings concerning functions of KIFs in this

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review. For a detailed discussion of earlier findings, readers may refer to the previous reviews [1,2,4–9].

Polarity of microtubules and properties of N-kinesin, M-kinesin, and C-kinesin

Microtubule is a polymer composed of α- and β-tubulins. It has a polarity with a fast growing end (usually called the "plus end") and a slow growing end (also called the "minus end"). In the nerve axon, microtubules are in a uniform polarity with the plus ends pointing to the synaptic terminal. In the proximal dendrite, the polarity of microtubules is mixed, while in the distal end, the plus ends point to the postsynaptic sites. In polarized epithelial cells, microtubules run apicobasally with their minus ends pointing to the apical surface. In most interphase cells, such as fibroblasts, microtubules radiate in various directions from the microtubule-organizing center (MTOC) at the cell centers near the Golgi apparatus. The minus ends of microtubules are at the MTOC and the plus ends point to the cell periphery.

"Conventional kinesin," the first member of the kinesin superfamily that was identified, consists of two 120-kDa heavy chains (KHCs) and two 64-kDa light chains (KLCs) [1,2,10]. When observed by the low-angle rotary-shadowing electron microscopy, it has a rodlike structure composed of

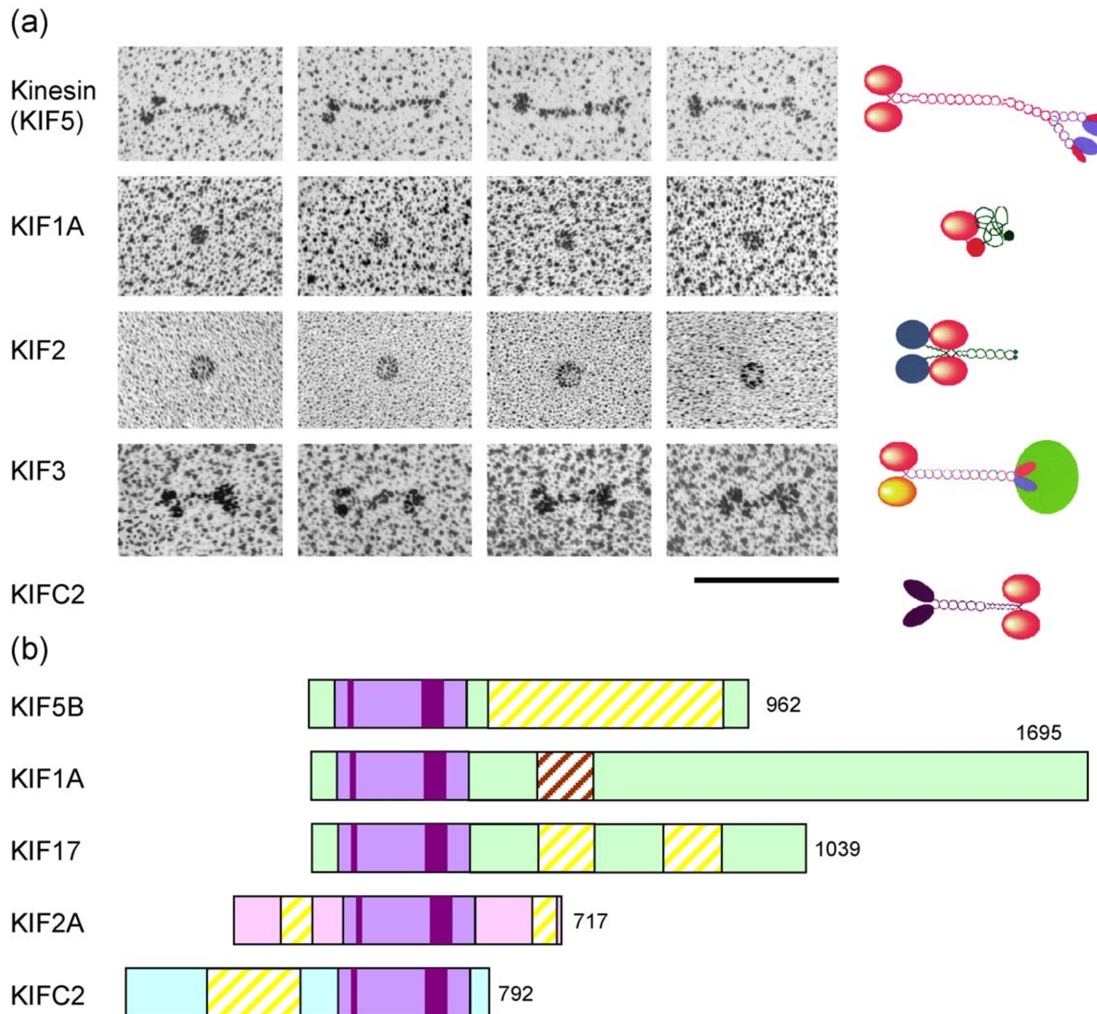


Fig. 1. Kinesin superfamily proteins (KIFs). (a) Left: main members observed by low-angle rotary-shadowing electron microscopy. Scale bar: 100 nm. Right: schematic illustration of the same KIFs based on electron microscopy studies or predicted from analyses of their primary structures. Red ovals represent motor domains. Kinesin is composed of a KHC (KIF5) dimer and KLCs (blue ovals) attached to the fanlike ends. KIF1A is monomeric. KIF2 forms a homodimer, and the motor domains are in the middle, that is, between the N-terminal regions (shown in blue) and the C-terminal regions (shown in green). KIF3 forms a trimer, a heterodimer of KIF3A and KIF3B associated with a soluble protein, kinesin-associated protein 3 (KAP3) (shown in green). KIFC2 also forms a homodimer, but its motor domain is on the opposite side. (Reproduced with permission from Ref. [1]). (b) Schematic representation of domain structures of some representative KIFs. There are 45 KIF genes in the murine and human genomes, which can be classified as N-kinesin, M-kinesin, and C-kinesin [12]. KIF5B, KIF1A, and KIF17 have their motor domains in the N-terminal and are classified as N-kinesin. KIF2 has its motor domain in the middle and is classified as M-kinesin. KIFC2 has its motor domain in the C-terminal and is classified as C-kinesin. The motor domains are shown in purple; thin red line, ATP-binding consensus sequence; thick red line, microtubule-binding consensus sequence. Yellow, hatched boxes represent the dimerization domains. The number of amino acids for each molecule is shown on the right.

two globular heads (10 nm in diameter), a stalk, and a fanlike end, with a total length of 80 nm (Fig. 1a) [10]. The globular heads are composed of KHCs, which serve as motor domains, while the KLCs constitute the fanlike end [10]. The motor domain is approximately 40 kDa, moves along microtubules and has ATPase activity.

A systematic molecular biological search of genes coding for proteins containing adenosine triphosphate (ATP)-binding and microtubule-binding consensus sequences led to the discovery of the kinesin superfamily proteins that participate in axonal transport [11]. Today, by combining molecular biological approaches with a Basic Local Alignment Search Tool (BLAST) search of proteins in public and private genome databases, a total of 45 KIFs have been identified in mouse and human genomes [12].

The 45 KIFs have been classified into three major types on the basis of the position of the motor domain: NH_2-terminal motor domain type, middle motor domain type, and COOH-terminal motor domain type (called N-kinesin, M-kinesin, and C-kinesin, respectively) (Fig. 1b) [2,12]. Most KIFs belong to N-kinesin, which are further classified into 11 classes. N-kinesin includes the KIF1, KIF3, KIF4, KIF5, KIF13, and KIF17 families as the major members. M-kinesin is composed of one class, namely, the KIF2 family. C-kinesin is composed of two classes, the KIFC1 and KIFC2/C3 families. KIFs of other species, including those of plants, could be classified under these 14 classes.

KIFs take various molecular shapes (Fig. 1a). KIF1A, KIF1Bα, and KIF1Bβ are monomeric; many KIFs form homodimers; some have associated proteins [1]. As discussed, conventional kinesin forms a heterotetramer consisting of 2 KHCs (corresponding to KIF5) and 2 KLCs. KIF3 forms a heterotrimeric complex, which is a heterodimer composed of KIF3A and KIF3B associated with a soluble protein called KAP3 (kinesin-associated protein 3).

From the results of in vitro motility assays, which have been carried out for many but not all KIFs, it appears that N-kinesins move towards the plus ends of microtubules, while C-kinesins move towards the minus ends of microtubules. M-kinesin has plus-end-directed motility and a unique microtubule-depolymerizing activity. In contrast, cytoplasmic dyneins are minus-end-directed motors. Each KIF has a characteristic velocity, and the velocity range of KIFs, as determined by in vitro motility assays, is mostly approximately 0.1–1.5 $\mu m\ s^{-1}$, which seems to closely correlate with the velocity observed in vivo for each KIF.

Although the motor domain is highly conserved among different KIFs, regions other than the motor domain are quite divergent and these regions serve as cargo-binding domain. The sequence diversity of the cargo-binding domains explains why KIFs transport a wide variety of cargoes, including membranous organelles and macromolecular complexes. Membranous organelles could be widely varied and may have a special lipid composition. Macromolecular complexes could also be widely varied and may contain mRNAs. However, each cargo appears to be selectively recognized and transported to precise destinations.

In short, microtubules have polarity and they are organized in special array within the cell. N-kinesin and M-kinesin appear to be plus-end-directed, and C-kinesin appears to be minus-end-directed. The motor domains are highly conserved, but outside the motor domain, each KIF has a unique sequence for the specific recognition of various cargoes.

Various types of KIF transport

We will summarize briefly various types of intracellular transport in which KIFs participate.

Axonal and dendritic transport

In the axons and dendrites, various cargoes are specifically transported by KIFs (Fig. 2a).

In the axons, precursors of synaptic vesicles are transported anterogradely by KIF1A and KIF1Bβ [5,11,13–15]. KIF1A and KIF1Bβ are products of separate genes; however, the C-terminal regions of KIF1A and KIF1Bβ have similarity, which probably explains the binding of both motors to synaptic vesicle precursors.

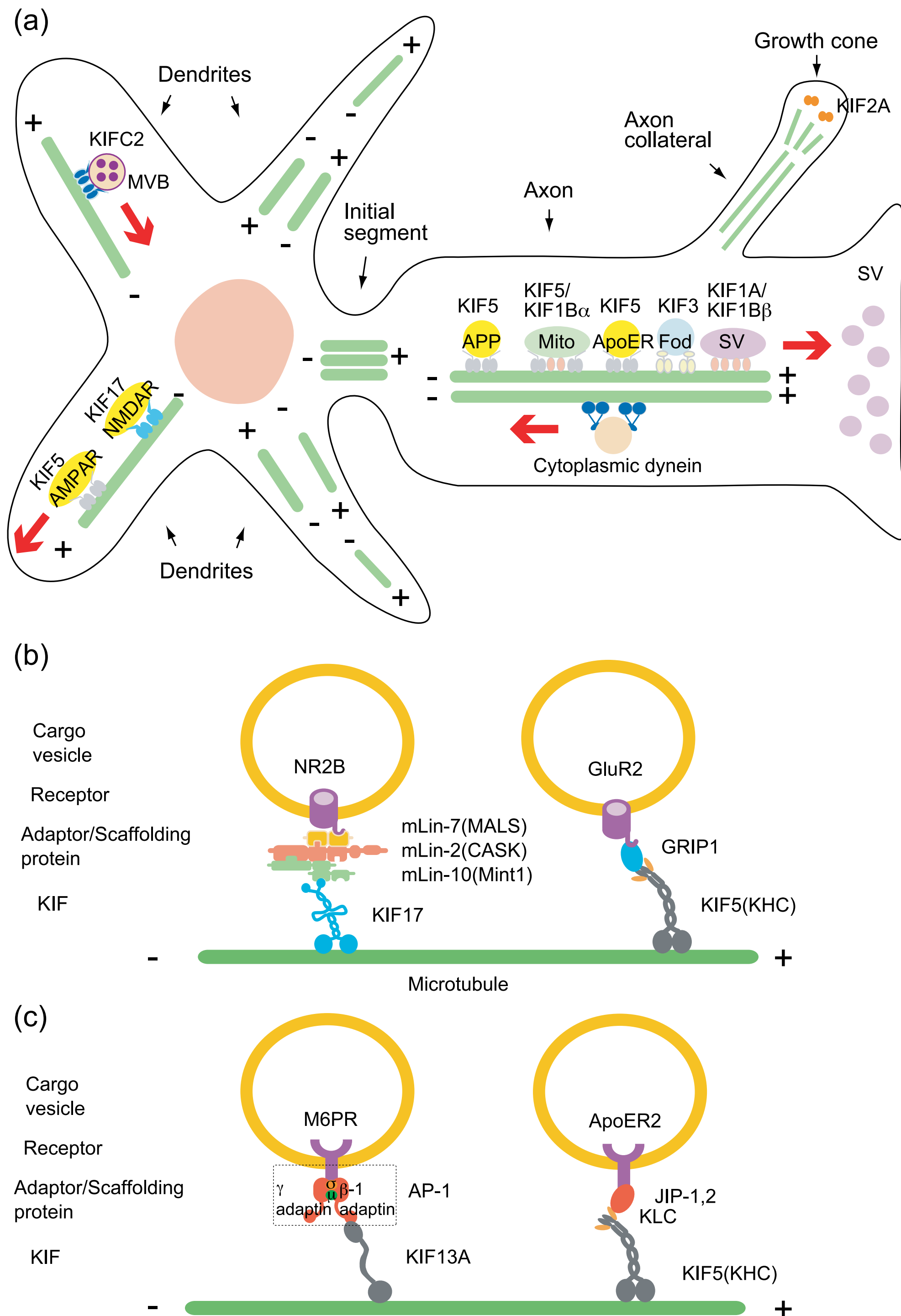
Knockout mice of KIF1A or KIF1Bβ show motor and sensory nerve defects and have a reduced number of synaptic vesicles at the synaptic terminals. In addition, a

Fig. 2. KIFs and their cargoes. (a) Scheme of KIFs and their cargo organelles in neurons. Motors and cargoes in axonal and dendritic transports are shown. On the right, the axon is shown. In the axon, microtubules (shown in green) are unipolar and the plus ends (+) point to the synaptic terminal. KIFs transport various cargoes from the cell body to the synapse; KIF1A and KIF1Bβ transport synaptic vesicle (SV) precursors, KIF3 transports vesicles associated with fodrin (Fod), KIF5 transports mitochondria (Mito) and vesicles containing ApoER2 or APP. Mitochondria are also transported by KIF1Ba. Cytoplasmic dyneins transport various cargoes in the opposite direction. KIF2A suppresses the elongation of axon collateral branch extension by its microtubule-depolymerizing activity at the growth cone. Microtubule bundles at the initial segment may function as cue for axonal transport for KIF5. Dendrites are shown on the left. In the dendrite, microtubules are of mixed polarity in the proximal regions, although it becomes unipolar at the distal regions. KIF5 transports vesicles containing AMPA-type glutamate receptors (AMPAR) and KIF17 transports vesicles containing NMDA-type glutamate receptors (NMDAR). In addition, KIC2 transports multivesicular body-like vesicles (MVB) toward the minus end of microtubules. Nucleus is shown in pink. (bc) Schematic representation of binding of KIFs to cargoes. In general, KIFs appear to bind to their cargoes through adaptor/scaffolding proteins. (b) Left: binding of NMDA receptor subunit NR2B-containing vesicles to KIF17 is mediated by a scaffolding protein complex containing mLin-7 (MALS), mLin-2 (CASK), and mLin-10 (Mint 1). Right: binding of AMPA receptor subunit GluR2-containing vesicles to KIF5 is mediated by a scaffolding protein GRIP1. KIF5 (KHC) binds directly to GRIP1, and the vesicle is transported to the dendrites. (c) Left: binding of mannose-6-phosphate receptor (M6PR)-containing vesicles to KIF13A is mediated by an adaptor complex, AP-1, which is composed of β1, γ, μ1, and s1 adaptin subunits. Right: Binding of ApoER2-containing vesicles to KIF5 is mediated by scaffolding proteins, JIP-1 and JIP-2. The binding of KIF5 to JIPs is mediated by KLC, not by KIF5 (KHC). In contrast to the GluR2 containing-vesicle, the vesicles are transported to the axons.

loss-of-function point mutation in the motor domain of KIF1B has been linked to a pedigree of a human hereditary peripheral neuropathy, Charcot–Marie-Tooth disease type 2A [15] (reviewed in Ref. [5]). This was the first clear indication that dysfunction of a motor protein is the cause of human peripheral neuropathy.

Vesicles containing amyloid precursor protein (APP), the precursor of the amyloid-β peptide that is deposited in the brains of Alzheimer's disease patients brain, are transported anterogradely in the axon by KIF5 [16,17]. The vesicles also contain β-secretase and presenilin-1, which cleave APP to yield amyloid-β peptide, and therefore, it is presumed that the cleavage of APP takes place in the vesicle. Vesicles containing ApoER2, the receptor for Reelin, which may function in neuronal development, are also transported anterogradely in the axon by KIF5 [18].

Vesicles associated with fodrin are transported by the KIF3 complex [19]. Mitochondria [20], lysosomes [21], and



tubulin oligomers [22] are transported by KIF5. Mitochondria are also transported by KIF1Bα [23], which is an alernative transcript of KIF1Bβ and has a shorter C-terminal tail.

In the dendrites, vesicles containing *N*-methyl-D-aspartate (NMDA)-type glutamate receptors are transported by KIF17 from the cell body to the postsynaptic sites [24,25]. That KIF17 plays an important role in memory has been demonstrated by the fact that transgenic mice overexpressing KIF17 show enhanced working or episode-like memory and spatial learning and memory [26]. Moreover, the gene expressions of KIF17 and NMDA receptor NR2B subunit appear to be coregulated with the potential involvement of a transcription factor, cAMP-response element binding protein (CREB), and its increased phosphorylation [25,26]. KIF17 belongs to N-kinesin and is a plus-end-directed motor, but specifically localized to dendrites.

Vesicles containing α-amino-3-hydroxy-5-methylisoxazoloe-4-propionic acid (AMPA)-type glutamate receptors are transported by KIF5 in the dendrites, from the cell body to the postsynaptic sites [27]. Because KIF5 could convey other cargoes to the axons as well, the cargoes can seemingly determine the destination in this case. This will be discussed later in more detail.

Multivesicular body-like organelles are transported by KIFC2 in the dendrites (Fig. 2a) [28]. It is specifically localized to the dendrites, but not in the axons. KIFC2 is presumably a minus-end-directed motor, although this is not shown directly.

mRNAs for an activity-regulated cytoskeleton-associated protein Arc and the α subunit of calcium/calmodulin-dependent protein kinase II (CaMKIIα), both of which play roles in long-term potentiation, may also be transported by KIF5 as macromolecular complexes [29].

Therefore, KIFs transport various molecules in the axons and dendrites with specificity. However, there is some redundancy in the specificity in terms of both the cargoes to be transported and KIFs. The specificity may be regulated by several mechanisms and both the motors and cargoes may play a part.

Intraflagellar transport and left–right axis formation

In addition to axonal transport, the KIF3 complex also participates in a specific transport called "intraflagellar transport (IFT)" [4,30,31]. In cilia or flagella, bundles of microtubules of specialized configurations, often described as "9 + 2" or "9 + 0" (9 doublets of microtubules with or without a central pair of microtubules), form the core. The microtubules in this configuration also have uniform polarity with the plus ends pointing towards the tips. The KIF3 complex transports protein components of cilia and flagella in a large macromolecular complex called IFT particles. The IFT particles are sufficiently large to occupy almost the narrow space between the plasma membrane and the core microtubule bundle and slide along the microtubules closely apposed to the membrane.

When $kif3b^{-/-}$ or $kif3a^{-/-}$ mice were analyzed, it was revealed that the knockout mice cannot properly establish the left–right axis [30,31] (reviewed in Ref. [4]). The reason was not clear at first. Upon further analysis, it was revealed that in $kif3b^{-/-}$ or $kif3a^{-/-}$ mice, the monocilia usually present on the node are not properly formed [30,31]. This led to the discovery of an unexpected role of nodal cilia in the establishment of the left–right axis. Formerly, it was assumed that the nodal cilia were immotile because of their unique "9 + 0" structure. However, high-resolution video microscopy revealed that in the wild-type embryos, the nodal cilia show a vortex movement that generates a leftward flow of extraembryonic fluid in the node [30,31]. Although it had been known that the node is the site where the symmetry of the embryo is first broken, the exact event had not been clear. It now seems that the leftward flow of the extraembryonic fluid in the node is a good candidate for the first event that breaks the symmetry of the embryo ("nodal flow theory") [4,30,31].

Vesicle trafficking and polarized epithelial transport

The mannose-6-phosphate receptor binds to mannose-6-phosphate recognition signals on the newly synthesized lysosomal enzymes. It has been shown that KIF13A, which is ubiquitously expressed in mice, has an essential function in targeting mannose-6-phosphate receptors from the *trans*-Golgi network to the plasma membrane [32].

In polarized epithelial cells, vesicles are targeted to the basolateral or apical domain. Although the targeting signals have been studied, it is not known how motors are involved in this process. KIFC3 is a recently identified KIF with a minus-end-directed motor activity, and is expressed in polarized kidney epithelial cells. The analysis has shown that it participates in the apical transport of Triton X-100-insoluble membrane organelles containing annexin XIIIb [33].

In addition, KIFC3 may also play a significant role in the positioning of the Golgi apparatus under cholesterol-depleted condition [34]. In cholesterol-depleted $kifc3^{-/-}$ mice, inwardly directed motility of the Golgi fragments is markedly reduced compared to that in cholesterol-depleted wild-type cells or cholesterol-replenished $kifc3^{-/-}$ mice.

Therefore, it is now clear that various KIFs are involved in many different types of intracellular transport, including axonal and dendritic transport, intraflagellar transport, polarized epithelial cell transport, and intracellular vesicular trafficking in general.

Cargo binding and differential sorting

Although the specificity of KIF transport has become increasingly clear, how it is achieved has not been clarified in molecular terms. Therefore, much effort has been spent on the search for the direct binding partners of KIFs in the past several years. The emerging concept from the obtained results

is a rather unexpected one: in many cases, KIFs appear to bind to their targets through adaptor/scaffolding complexes.

One of the first examples of this paradigm is the binding of KIF13A to the mannose-6-phosphate receptor [32]. The binding of KIF13A to the mannose-6-phosphate receptor has been found to be mediated by an adaptor complex AP-1 [32], which functions in the vesicle trafficking from the *trans*-Golgi network to the plasma membrane and is composed of β1-, γ-, μ1-, and σ1-adaptin subunits. It was shown that the tail region of KIF13A binds directly to the ear domain of β1-adaptin (Fig. 2c, left) [32]. Direct binding of the mannose-6-phosphate receptor to AP-1 had been shown previously, therefore, AP-1 mediates binding of KIF13A to the mannose-6-phosphate receptor [32].

The next example is KIF17 that transports vesicles containing the NMDA receptor [24]. Here, it was shown that KIF17 binds to the NMDA receptor subunit NR2B via a scaffolding complex composed of three PDZ-domain-containing proteins, mLin-10 (Mint 1), mLin-2 (CASK), and mLin-7 (MALS). The tail region of KIF17 interacts directly with a PDZ domain of mLin-10 (Fig. 2b, left) [24].

In the case of KIF5, many cargoes are bound via KLC. KLC contains tetratricopeptide repeat (TPR) motifs, and it was shown that KLC binds to c-jun NH_2-terminal kinase (JNK)-interacting proteins (JIPs), JIP-1, JIP-2, and JIP-3 (also called *sunday driver* and JSAP) [18,35], which are scaffolding proteins for the JNK signaling pathway and bind kinases. JIP-1 and JIP-2 are related in sequence, but JIP-3 is not. JIP-1 and JIP-2 had been previously shown to bind directly to ApoER2, the receptor for the Reelin ligand, and it was confirmed that KIF5 coprecipitates with JIP scaffolds and ApoER2 [18]. Therefore, it appears that the binding of vesicles containing ApoER2 and KIF5 is mediated by JIP scaffolding proteins and KLC (Fig. 2c, right). The binding partner of JIP-3 is currently less clear [35].

KIF5 transports vesicles containing APP as well and in this case, the binding of KIF5 and vesicles is also mediated by TPR motifs of KLC [16,17]. Although it was originally considered that the TPR motif of KLC directly binds to an integral membrane protein, APP, it now seems that the binding of KLC and APP may also be mediated by JIP scaffolding proteins [36,37].

In some species such as fungi, KLCs are absent, and it has been speculated that KHCs alone should be sufficient for binding to some cargoes. In agreement with this expectation, it was found that vesicles containing AMPA receptors bind to KHC [27]. The binding of KHC to the vesicles is mediated by glutamate receptor-interacting protein (GRIP1), another scaffolding protein containing several PDZ domains [27]. KHC binds directly to GRIP1, which then binds to the GluR2 subunit of AMPA receptors (Fig. 2b right). The binding site of KHC with GRIP1 overlaps with the cargo-binding domain of the fungus kinesin [27].

The transport of macromolecular complexes containing mRNAs in the dendrite also appears to be mediated by KHC [29]. Therefore, it is suggested that when cargoes are bound via KHC at the binding site common to fungus kinesin, they may be transported to dendrites.

The above findings suggest that whether cargoes are bound by KLC or KHC may determine the sorting. However, KIF17 is preferentially localized to dendrites. Then, does the KIF5 motor itself prefer a particular transport direction? Before considering this aspect, it is probably worth noting some of the morphological differences between dendrites and axons. First, there are usually several dendrites, but there is only one axon. Dendrites usually have a large diameter near the cell body, which gradually tapers off. In contrast, axons usually have uniformly small diameter throughout. Therefore, if one assumes that the diameter of an axon is one tenth that of the cell body, axonally transported materials need to be targeted to the area only 0.25% of all directions [38].

It has been shown recently that the KIF5 motor itself may prefer sorting to the axon. KIF5 binds preferentially to microtubules in the initial segment of axons [38]. The initial segment of the axons, which is the region involved in initiating the action potential, has some unique morphological characteristics. Its plasma membrane has thick associated structures underneath and it has a unique tightly packed bundle of microtubules (Fig. 2a). The biochemical background leading to this unique arrangement of microtubules is not completely understood, but it was shown that the microtubules in this region have high affinity to EB-1 [38], which is known to bind to the tips of growing microtubules. Therefore, KIF5 may also recognize the same property as that recognized by EB-1. In any event, the results suggest that microtubules at the initial segment may serve as a directional cue for polarized axonal transport [38].

The same study showed an interesting feature of transport observed in the cell body. Previously, it was difficult to analyze the transport within the cell body at high spatial and temporal resolution, but an elegant and sophisticated application of critical-angle fluorescence microscopy enabled a hitherto unperformed analysis [38]. It was shown that tubulovesicular carriers of various shapes are highly motile with long processivity and directly move from the *trans*-Golgi network to the axon, while carriers to dendrites are vesicular in shape, less motile with short processivity and evenly distributed within the cell body [38]. These differences may also be related to the mechanisms underlying the differential sorting achieved by KIFs.

It is clear therefore that multiple mechanisms may work together to accomplish the selective sorting of functional molecules towards axons and dendrites.

In polarized epithelial cells, KIFC3 transports cholesterol-rich, apically targeted vesicles containing hemagglutinin and annexin XIIIb, a marker for the apically transported vesicles [33]. The dynein motor does not associate with the vesicles, and overexpression of KIFC3 and its dominant-negative form revealed that KIFC3 is necessary for the apical transport of annexin XIIIb [33]. Therefore, KIFs may have yet another

previously unrecognized function, that is, its involvement in vectorial transport in polarized epithelial cells.

In short, KIFs appear to bind their cargoes via adaptor/scaffolding proteins, and both the intrinsic properties of KIFs and the manner by which KIFs bind to their cargoes may determine the sorting route. KIFs are involved not only in the selective transport in neurons, but also in that in polarized epithelial cells. As more is learned about the molecular details of KIFs binding to cargoes, more will be learned about the mechanisms of sorting and selective transport that occur in neurons as well as in other cell types.

Neuronal morphology

During neuronal development, postmitotic neurons develop primary axons that extend towards their targets, while other collateral branches remain short. The processes of extension and/or suppression of collaterals are essential for correct wiring of the brain: however, mechanisms underlying its regulation are not fully understood.

Through the analysis of knockout mice of KIF2A, a unique middle motor domain type KIF abundantly expressed in juvenile neuron and concentrated in growth cones [11,39], it was found that KIF2 has an unexpected function in vivo, that is, the suppression of axon collateral branch extensions [40]. *kif2a*$^{-/-}$ mice show multiple brain abnormalities including laminary defects and mispositioning of nerve nuclei. Morphological observation suggested that axonal morphology is abnormal in the *kif2a*$^{-/-}$ brain; in the cerebral cortex of the *kif2a*$^{-/-}$ mice, greater numbers of axon collaterals run horizontally compared to those in the cerebral cortex of wild-type mice [40]. This implies that either an increased number of collateral is formed or an increased elongation of collaterals occurs in the *kif2a*$^{-/-}$ mice. To determine which of the two possibilities occurred, neurons from both mice were cultured for further analysis. *kif2a*$^{-/-}$ neurons formed aberrantly elongated collateral branches, which further branch into tertiary and quaternary branches (Figs. 3c and d) [40]. In contrast, the elongation of collaterals is basically suppressed in the wild-type neurons. The average number of collaterals was the same for both the wild-type and *kif2a*$^{-/-}$ neurons, suggesting that the elongation, but not the formation, is affected in the *kif2a*$^{-/-}$ mice. Observation of neuronal migration for a long time revealed that the migration defect of kif2a$^{-/-}$ neurons could be caused at least partially by the uncontrolled elongation of axon collaterals [40].

Further analysis revealed that this is attributable to the microtubule-depolymerizing activity of KIF2A. First, the fluorescence loss in photobleaching showed that microtubule-depolymerizing activity is decreased in the growth cones of *kif2a*$^{-/-}$ neurons. Next, when the behavior of individual microtubules is observed, a fundamental difference is noted between wild-type and *kif2a*$^{-/-}$ cells. In the wild-type cells, when the tips of the microtubules reach the cell edge, the microtubules stop growing. In contrast, in the *kif2a*$^{-/-}$ cells, the microtubules continue to grow [40]. Taken together, it is suggested that in the *kif2a*$^{-/-}$ growth cone, the microtubule-depolymerizing activity is not fully operational and the microtubules keep elongating, resulting in the overextension of collateral branches (Figs. 3a and b) [40]. Thus, KIF2A is essential for brain wiring.

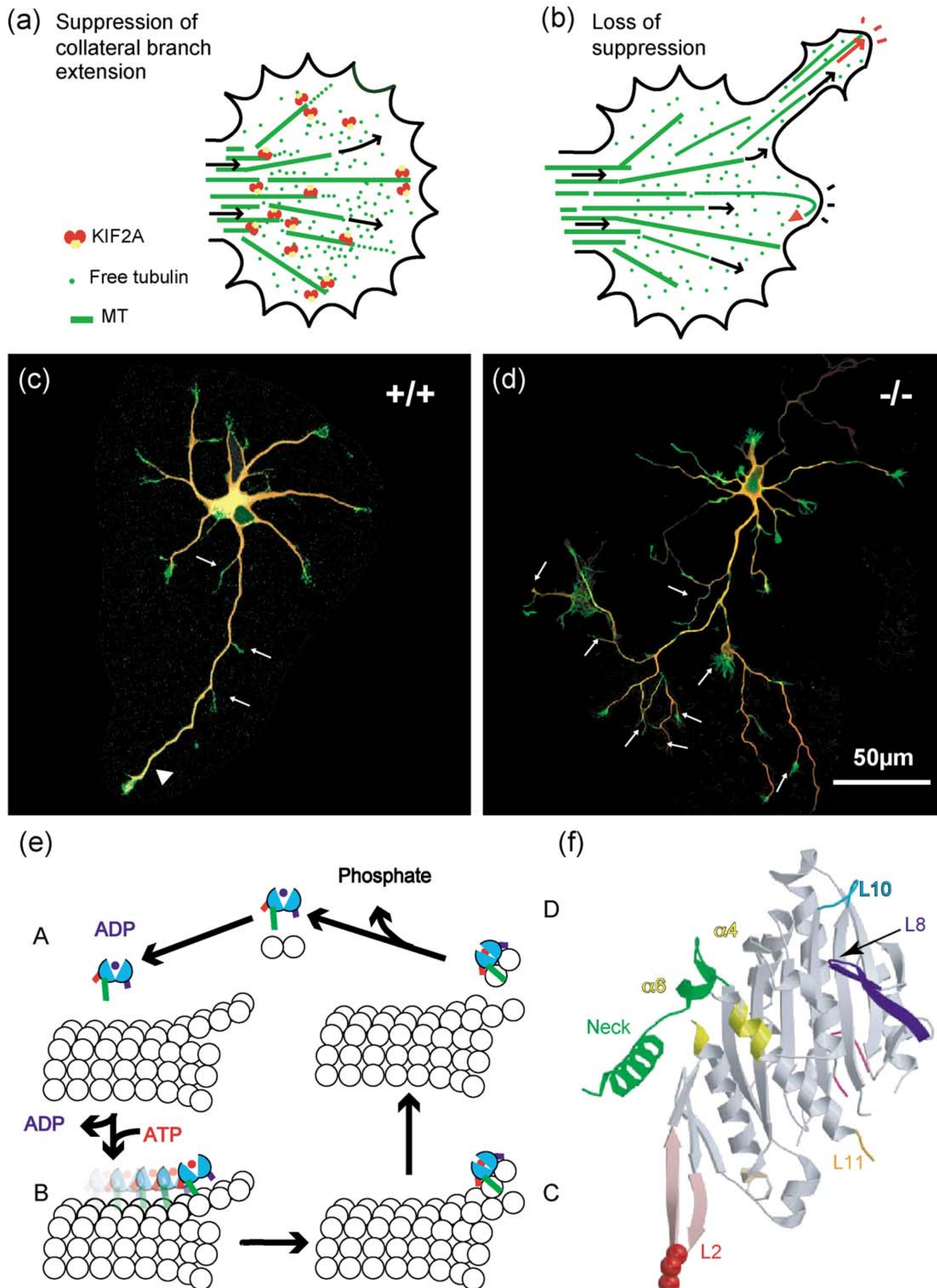
The KIF2 family belongs to M-kinesin and has a motor domain in the middle of the molecule. Presumably, the position of the motor domain is related to its unique microtubule-depolymerizing activity, but the reason for this has not been previously clarified. Recently, X-ray crystallography analysis of KIF2C revealed the structural correlation of its unique class-specific microtubule-depolymerizing activity [41]. Three principal structural features underlying the microtubule-depolymerizing activity are the unique N-terminal neck helix, the loop L2, and the loop L8 (Figs. 3e and f). The class-specific N-terminal neck of KIF2C adopts a long and rigid helical structure that extends vertically into the interprotofilament grooves (Fig. 3e) [41]. Because of this neck helix, KIF2C cannot bind tightly to the sidewall of the microtubule. The nucleotide-binding pocket is trapped in the open state, and thus, the ATP-bound KIF2C diffuses along the microtubule (Fig. 3e [B]). When KIF2C reaches the end of microtubules, where protofilaments have a leeway to curve, the curved conformation allows the full contact of KIF2C to the microtubule (Fig. 3e [C]). This contact induces

Fig. 3. Suppression of collateral branch extension by KIF2 and structural model of the mechanism of microtubule depolymerization by M-kinesin. (a and b) Proposed model of suppression of collateral branch extension by KIF2A. (a) In the presence of KIF2A, growth cone extension is suppressed because microtubules maintain a dynamic equilibrium between polymerization and depolymerization, particularly at the edge of the growth cone. (b) In the absence of KIF2A, microtubule tips at the cell edge do not show controlled dynamics and hit the membrane. Microtubule tips turn back (red arrowhead) or push the membrane forward (red arrow). Therefore, growth cones are released from collateral suppression and begin to extend. (c and d) Representative hippocampal neurons 2 days after plating. Neurons were double-labeled with FITC-conjugated phalloidin for F-actin (green) and anti-tubulin antibody (red). *kif2a*$^{-/-}$ neurons extend longer collaterals (arrows in [d]) than *kif2a*$^{+/+}$ neurons (arrows in [c]). (ad, reproduced with permission from Ref. [40]). (e) Structural model of the mechanism of microtubule depolymerization by M-kinesin (KIF-M). (A) ADP bound KIF-M (light blue) binds to the sidewall of the microtubule. (B) The neck helix (green) interferes with the M loop in the interprotofilament grove, and KIF-M cannot bind tightly to the sidewall of the microtubule. The nucleotide-binding pocket is trapped in the open state. Thus, ATP-bound KIF-M diffuses along the microtubule protofilament. (C) When KIF-M reaches the end of the microtubule, the curved conformation of the protofilament allows full contact with KIF-M. The L8 loop (purple) closes the nucleotide-binding pocket and ATP hydrolysis takes place. The neck helix destabilizes the lateral interaction of the protofilament, and the loop 2 KVD finger (red) stabilizes the curved conformation of the interdimer groove. (D) Tubulin dimer or oligomer is spontaneously released from the curved end of the protofilament. Hydrolysis of ATP on the tubulin dimer (or oligomer) releases KIF-M and the next cycle starts. (f) Structure of KIF2C (AMP-PNP-bound form). View from the microtubule-binding side. The clusters of the KIF-M-specific residues are indicated by coloring. The KIF-M-specific KVD motif, the residues Lys293-Asp295, is shown by red spheres. (e and f, reproduced with permission from Ref. [41]).

the loop L8 to close the nucleotide-binding pocket, which triggers the ATP hydrolysis. The neck helix destabilizes the lateral interaction between the protofilaments, and the loop L2, which forms a unique fingerlike structure, sufficiently long and rigid to reach the next tubulin subunit, stabilizes the curved conformation of the interdimer groove (Fig. 3e [C]). The tubulin dimer or oligomer is spontaneously released from the curved end of the protofilament (Fig. 3e [D]) [41]. Thus, the combination of three unique structures contributes to the microtubule-depolymerizing activity of KIF2 motors.

Mechanism of motility

Although KIF1A and KIF1B are monomers, in vitro motility assay shows that they move processively. Most



KIFs function as dimers, and therefore, hand-over-hand movement is achieved. The unique properties of KIF1A and KIF1B make them ideal models for solving the mechanism of KIF motility.

Recently, KIF1A has been shown to move by biased Brownian movement using single molecule biophysics [42] and the optical trapping system [43]. First, it was demonstrated that a single ATP hydrolysis triggers a single stepping movement of the KIF1A monomer. The step size was distributed around the multiples of 8 nm, which corresponds to the periodicity of the binding site on the microtubule. Further analysis then showed that the movement can be explained by the following model [43]. First, let us start from the ATP binding state of KIF1A, when it is tightly bound to microtubules. Upon hydrolysis of ATP to ADP, KIF1A is released from the binding site. There is no net movement in this step. Then, the ADP-bound form of KIF1A moves along the microtubule by one-dimensional Brownian movement. When ADP is released, KIF1A binds again to the microtubule, and in this step, KIF1A has bias in the selection of the binding site toward the plus end by approximately 3 nm on average.

Comparison of X-ray crystallography of KIF1As with different ATP analogues, each representing a distinct transitional energy state, further revealed how chemical energy is converted to mechanical movement [44]. The loops L11 and L12 form the main microtubule-binding elements of KIF1A [45,46] and these two elements are used alternately for microtubule binding. In the ATP-bound state (AMPPNP-bound form), L11 is extended and interacts with the H11' helix of tubulin, and KIF1A and the microtubule are tightly bound. In this state, L12 is extended upwards and away from the microtubule. However, in the hydrolysis step of ATP (ADP-vanadate form), both L11 and L12 are raised from the microtubule and this results in the active detachment of KIF1A from the microtubule. After phosphate release, it becomes the ADP form and the flexible L12 is extended downwards onto the tubulin C-terminal E-hook. This allows the one-dimensional diffusion in a weakly binding state. In this manner, KIF1A uses two microtubule-binding loops alternately [44].

Structural similarities between kinesin, myosin, and G proteins indicate that other molecules may universally utilize this kind of strategy.

Conclusion

KIFs utilize microtubules as rails and participate in various intracellular transports, such as axonal and dendritic transport, selective transport in polarized epithelial cells, intraflagellar transport, and general membrane trafficking. Although the motor domains of KIFs that have microtubule- and ATP-binding sites are highly conserved, KIFs take various molecular forms and bind to diverse cargoes including membranous organelles, macromolecular complexes, and mRNAs. The association of cargoes and KIFs tends to be mediated by adaptor/scaffolding proteins. Many cargoes are transported selectively to precise locations. Mechanisms to achieve this precise transport need to be elucidated further, but it is suggested that both the KIFs themselves and how the KIFs associate with cargoes are the determinants.

KIF functions underlie some diseases, and they are also involved in development. A recent unexpected finding is that KIF2A, an M-kinesin, functions in neuronal development by suppressing collateral axonal branch extension through its unique ability to depolymerize microtubules at their ends. Recent studies based on wide range of molecular cell biology and molecular genetics are emergingly revealing that KIFs are fundamental not only for various cellular functions, but also for developmental events such as left–right determination and brain wiring and further for higher brain functions such as memory and learning.

X-ray crystallography cryoelectron microscopy and single molecule biophysics have revealed the structural features underlying the motility or microtubule-depolymerizing activity of KIFs.

In conclusion, these multiple approaches in analyzing structures, functions, and dynamics of KIFs will illuminate many basic mechanisms underlying intracellular transports and will be a very promising and fruitful area for future studies.

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