Intracellular Transport and Kinesin Superfamily Proteins, KIFs: Structure, Function, and Dynamics

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functions such as memory and learning and activity-dependent neuronal survival during brain development, and for the determination of important developmental processes such as left-right asymmetry formation and suppression of tumorigenesis. Accumulating data have revealed a molecular mechanism of cargo recognition involving scaffolding or adaptor protein complexes. Intramolecular folding and phosphorylation also regulate the binding activity of motor proteins. New techniques using molecular biophysics, cryoelectron microscopy, and X-ray crystallography have detected structural changes in motor proteins, synchronized with ATP hydrolysis cycles, leading to the development of independent models of monomer and dimer motors for processive movement along microtubules.

I. INTRODUCTION

Cells have developed a differentiated delivery system to sustain their specific functions and morphology. This intracellular transport mechanism is spatially and temporally controlled by microtubule-dependent motor proteins. As shown by recent data, the basic principles of intracellular transport are highly conserved, and motor proteins constitute a common molecular machinery for intracellular transport in neurons as well as in other types of cells (65, 71, 96, 101, 229). Compared with other cell types, in which only a short distance of transport is required to reach the destination, neurons with long neurites have a well-developed transport system. Indeed, the transport of membrane organelles in axons can be directly observed by high-resolution optical microscopy, which reveals that different-shaped membrane organelles are transported at different speeds with different directions. Thus axonal transport and dendritic transport serve as a good model system for elucidating one of the fundamental mechanisms of sustaining life in organisms.

The directionality of transport is determined by interactions between motor proteins and the microtubule rails, tubular structures 25 nm in diameter composed of heterodimers of α- and β-tubulins (64, 65). A microtubule has its own direction with plus and minus ends: microtubules polymerize faster at the plus end than at the minus end, which is less dynamic. Each motor protein senses the direction of the microtubules and steers with its own directionality toward the determined end. Thus, to understand each type of transport, knowledge about the directionality of microtubules within cells is necessary. In axons, microtubules are unipolar, and the plus ends always point to the periphery. Therefore, anterograde motors, which drive transport from the cell body to the cell periphery in axons, are necessarily plus-end-directed, whereas retrograde motors, which drive transport from the periphery to the cell body, are minus-end-directed. However, the directionality of microtubules is mixed in proximal dendrites, in which both types of motor can work (6, 18).

The polarity of microtubules also depends on the cell type. For example, in epithelial cells, the minus ends of microtubules are directed towards the apical surface. In fibroblasts, microtubules radiate in various directions from the microtubule-organizing center near the nucleus, and their plus ends are directed towards the periphery. Each type of cell has its own pattern of highly organized microtubule rails and uses compatible motors along them.

In the mid 1980s, two representative motors, conventional kinesin and cytoplasmic dynein, were purified from the brain and found to utilize ATPase activity to drive microtubule plus-end- and minus-end-directed transport, respectively (15, 172, 230). Initially, it was thought that these two motors could accomplish most of the bidirectional transport in cells. In contrast, since then, a range of cargoes steered by microtubule motors have been identified and characterized (65).

Quick-freeze, deep-etch electron microscopy of axons revealed fine structures associated with membrane organelles and microtubules at a very high resolution. Short crossbridges can be detected between membrane organelles and microtubules, and these are supposed to correspond to the molecular motors (Fig. 1) (64–66, 68). Indeed, these crossbridges have different shapes, reflecting the variable shapes of molecular motors (Fig. 1, A–C). In 1992, the first molecular biological search of a mouse brain cDNA library identified a group of 10 molecular motor genes, which were then designated as kinesin superfamily proteins (KIFs) (1).

Over the course of evolution, various combinations of dynein subunits have come to constitute a large “dynein complex” for the purpose of binding a variety of cargoes (191, 231), while the number of kinesin family members has increased to provide variation. Either way, each molecular motor has attained the specificity to bind to its partner on the cargo complex, enabling the proper execution of a transport process based on a strict regulation mechanism.

In this review, we focus on the intracellular transport mediated by KIF motor proteins to understand the molecular mechanism underlying the regulation of differentiated transport. Thus we do not refer to mitotic KIF motors in this review; they have been discussed comprehensively in other reviews (133, 141, 198).

This review is composed of three parts. First, each type of molecular transport mediated by KIF motors is described in terms of the route taken, the particular cargoes being transported, and the particular motor proteins involved (see sects. III–VII). At a higher level, such as in tissues and individual animals, motor proteins have been
implicated in determining cell morphology, cell-cell contacts, and differentiation (see sect. VIII).

Second, the interactions among molecules are discussed. To execute multistage transport processes, intermolecular interactions between motor proteins and cargoes are indispensable (see sect. IX). Third, the intramolecular mechanism underlying the conformational change in motor proteins is also discussed, through an analysis of the crystal structures and the cryo-electron microscopic (EMs) images of motor proteins (see sect. X).

II. CLASSIFICATION

KIFs possess a conserved globular motor domain, which involves an ATP-binding sequence and a microtubule-binding sequence (Fig. 2) (65, 68, 71). This globular motor domain, called the “head,” hydrolyzes ATP and transfers chemical energy to result in the motility of each KIF along microtubules with intrinsic directionality. While motor domains show high amino acid sequence homologies of ~30–60% among various KIFs, other regions, including a filamentous “stalk” region and a globular “tail” region, are quite variable (65, 68). Generally, motor proteins use their stalk regions to dimerize with each other. However, some KIFs have a short coiled-coil region and exist as monomers, or some form heterodimers among the subfamily members. KIFs bind to cargoes through their variable tail regions (65, 68). Some accompany light chains or associated proteins to bind indirectly to cargoes (Fig. 3). In addition to transporting cargoes, motor proteins bind to chromosomes and spindles and are functional during mitosis and meiosis. Some motor proteins participate in both intracellular transport and mitosis.

KIFs can be broadly grouped into three types depending on the position of the motor domain within a molecule. N-kinesins have a motor domain in the NH$_2$-terminal region, M-kinesins have one in the middle, and C-kinesins have theirs in the COOH-terminal region (Fig. 2). The intramolecular position of the motor domain grossly determines the directionality of the motor. While N-kinesins drive plus-end-directed motility, C-kinesins minus-end-directed motility.
Recently, all KIF genes in the mammalian and human genomes have been systematically identified (137). There are a total of 45 KIF genes in the mouse genome, 38 of which are expressed in the brain. Considering that alternative splicing can produce two to three mRNAs from each gene, with different tail domains that bind to different cargoes, the number of KIF proteins is approximately twice the number of KIF genes, perhaps even larger. Each KIF protein has been identified independently and named by different criteria, so there have been reported hundreds of KIFs containing the same KIFs called with different names, which has caused confusion and miscommunication among researchers. To improve the situation, a standard kinesin nomenclature was established in 2004, which classified them into 14 families according to the results of phylogenetic analyses (24, 68, 118, 119, 135, 136). Of the 14 families, only one contains M-kinesins, and only one contains C-kinesins; the remaining 12 families comprise N-kinesins, a bias that can be explained by the fact that KIFs usually drive anterograde transport while most retrograde transport is mediated by cytoplasmic dynein. In spite of the new nomenclature, to date, its usage has only been limited to introduce rough categories of the motors. Indeed, each KIF is still called by its original name, since different KIFs usually transport different cargoes, even if they are categorized in the same group. The KIFs implicated in transporting identified cargoes are shown in Figure 2, according to the new classification.

III. ANTEROGRADE AXONAL TRANSPORT

A. KIF1A/Unc-104: A Monomeric Motor for Synaptic Vesicle Precursor Transport

Among the KIFs (Fig. 2), some members; KIF1A, KIF1Bα, and KIF1Bβ, have a short coiled-coil domain in the stalk region, which are supposed to exist as monomers, the number of KIF proteins is approximately twice the number of KIF genes, perhaps even larger. Each KIF protein has been identified independently and named by different criteria, so there have been reported hundreds of KIFs containing the same KIFs called with different names, which has caused confusion and miscommunication among researchers. To improve the situation, a standard kinesin nomenclature was established in 2004, which classified them into 14 families according to the results of phylogenetic analyses (24, 68, 118, 119, 135, 136). Of the 14 families, only one contains M-kinesins, and only one contains C-kinesins; the remaining 12 families comprise N-kinesins, a bias that can be explained by the fact that KIFs usually drive anterograde transport while most retrograde transport is mediated by cytoplasmic dynein. In spite of the new nomenclature, to date, its usage has only been limited to introduce rough categories of the motors. Indeed, each KIF is still called by its original name, since different KIFs usually transport different cargoes, even if they are categorized in the same group. The KIFs implicated in transporting identified cargoes are shown in Figure 2, according to the new classification.

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mers. Among them, KIF1A is a brain-specific microtubule motor, with 1,695 amino acid residues (Fig. 2) (1, 168). It was first identified as Unc-104 in Caenorhabditis elegans, the mutation of which causes a deficiency of synaptic vesicles in axons (53). A unique property of KIF1A is its existence as a monomer, which is strongly suggested by various experimental data (168). Sucrose velocity gradient, native polyacrylamide gel electrophoresis (PAGE), and differential laser light scattering analyses have shown that the native molecular mass of KIF1A in solution is 200 kDa, which closely agrees with the molecular mass of 190 kDa estimated from its amino acid sequence. Low-angle rotary-shadowing electron microscopy also revealed that KIF1A is a single globular molecule (Fig. 3).

A motility assay using microtubules demonstrated that KIF1A moves towards the plus ends of microtubules at a velocity of 1.2 μm/s (168), which suggests that KIF1A is one of the fastest anterograde motors. Nerve ligation experiments showed the accumulation of KIF1A in the proximal region of a ligated site where anterogradely transported membrane organelles accumulated. Further experiments using immunocytochemistry and immunoprecipitation revealed that KIF1A-containing vesicles correspond to a particular population of synaptic vesicle precursors that contain synaptophysin, synaptotagmin, and Rab-3A, but do not contain plasma membrane proteins, such as syntaxin1A or SNAP25 (168) (Fig. 4). Evidence that conventional kinesin (KIF5, see sect. III D) and KIF3 (see sect. II E) are not present in KIF1A-containing vesicles suggests independent transport of these three motor proteins in axons.

Recent work has shown that KIF1A binds to its cargo through its pleckstrin homology (PH)-domain in the tail region, which interacts specifically with phosphatidylinositol 4,5-bisphosphate (PIP2) (109) (Fig. 5). When KIF1A molecules formed a cluster at the PIP2-abundant membrane surface, the efficiency of the motor activity of KIF1A increased (223). The authors of this report also showed that recombinant chimeric KIF1A, which is designed to dimerize, transports vesicles as efficiently as highly concentrated monomeric KIF1A. In another report, recombinant KIF1A of higher concentration was detected to exist as a dimer even in the soluble condition. They insisted that presence of the short coiled-coil region is sufficient to dimerize KIF1A (201). However, these data do not directly indicate that native monomeric KIF1A molecules dimerize when clustered on the membrane. Moreover, a newly identified binding partner, liprin-α, colocalized with KIF1A by binding to the coiled-coil region of KIF1A (202). The degree of contribution of dimeric KIF1A to molecular transport will need to be determined in the future.

Functional and biological damage caused by disruption of kif1A has been reported in mouse (251). Mutant mice were born alive, but all died within 24 h because they did not suckle milk. Severe motor and sensory abnormalities were explained by a decrease in the number of synaptic terminals per unit area, and in the density of synaptic vesicles at synaptic terminals to 50–60% of that seen in wild-type mice (Fig. 7). Focal neuronal death was also observed in several brain areas. The premature death of primary cultured hippocampal neurons within 13 days

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**Fig. 4.** Intracellular transport by molecular motors in neuronal cells.
coincides well with the beginning of the synthesis of KIF1A in wild-type neurons (251).

Although KIF1A is vital for neuronal function and survival, its functions are partially compensated for in knockout mice; about half of the synaptic vesicles density is still observed in knockout mice. This observation suggests the existence of complementary motor proteins, which is often the case with the intracellular transport system.

B. KIF1B: A Second Monomeric Motor for Transport of Synaptic Vesicle Precursors

KIF1B is an isoform generated by alternative splicing of the gene encoding KIF1A. The two motor proteins have identical motor domains, but completely different COOH-terminal tail regions (Fig. 2), indicating their involvement in the transport of different cargoes, because motors bind to cargoes through their tail regions. Biochemical analyses such as glutathione S-transferase (GST)-pulldown assays and vesicle immunoprecipitation using an anti-KIF1B-specific antibody have revealed that KIF1B transports synaptic vesicle precursors containing synaptotagmin, synaptophysin, and SV2 (254), suggesting a partially overlapping function of KIF1B with that of KIF1A (Fig. 4). When the function of KIF1B was investigated in kif1B knockout mice, in which neither KIF1Bα nor KIF1Bβ was expressed, mice were born alive but died within 30 min due to apnea. No significant defects were seen in the structures of alveoli, respiratory muscle, or neuromuscular junctions involved in respiration, suggesting the possibility that the apnea originated from neurological damage. In the brains of kif1B knockout mice, the number of neuronal cell bodies was <25% of that in the controls. Neuronal loss in the respiratory center was so severe that it was likely to cause neonatal apnea. The density of synaptic vesicles also decreased to 50–60% of that in controls.

When hippocampal neurons obtained from kif1B knockout embryos were cultured, a significant proportion of mutant cells died within 1 wk of plating (254), similar to kif1A null cells. Because exogenous expression of KIF1Bβ but not that of KIF1Bα rescued the neuronal death, KIF1Bβ is suggested to be mainly responsible for the mutant phenotype. Unlike KIF1Bβ, the absence of KIF1Bα in kif1B knockout mice seems to be compensated by other motors.

Although kif1B knockout mice died shortly after birth, heterozygotes survived. After 1 year, however, they come to show progressive muscle weakness and motor uncoordination (254) (Fig. 7). In the peripheral axons of heterozygous mice, a specific decrease in the levels of KIF1Bβ and synaptic vesicle proteins, such as synaptotagmin and SV2, was observed.

After gene mapping of murine kif1Bβ on chromosome 4E, the Charcot-Marie-Tooth disease type 2A (CMT2A) was mapped to the overlapping human chromosome region, 1p35–36. Analysis of a pedigree of CMT2A revealed a Q-to-L (glutamine-to-leucine) mutation in the consensus ATP-binding site of the KIF1B motor domain in heterozygote patients; this mutation causes a significant decrease in microtubule-dependent ATPase activity in vitro (254). Exogenous expression of the Q-to-L mutant KIF1Bβ in fibroblasts also caused perinuclear aggregation of the motor proteins instead of their transport to the peripheral plus ends of microtubules, suggesting kif1Bβ as a causative gene for Charcot-Marie-Tooth disease type 2A.

C. KIF1Bα: A Monomeric Motor for Mitochondrial Transport

KIF1B, another member of the kinesin-3 family, was identified in 1994 and is now called KIF1Bα (Fig. 2) (153). Experimental data suggest that KIF1Bα also exists as a monomer, and by low-angle rotary-shadowing electron microscopy, KIF1Bα is observed as a single-head globular molecule, sometimes with a short tail (Fig. 3). Immunocytochemistry and subcellular fractionation data showed colocalization of KIF1Bα and mitochondrial markers. An in vitro reconstruction assay succeeded in visualizing the...
cotransport of KIF1Bα and purified mitochondria along microtubules at a velocity of 0.5 μm/s, suggesting that KIF1Bα is the motor involved in the transport of mitochondria (153) (Fig. 4). As hinted at in the previous section, KIF1Bα is not the only motor involved in the transport of mitochondria. Knockout of KIF5B or KIF5C, two closely related subtypes of conventional kinesin (100, 215), showed that KIF5 molecules are also motors for mitochondrial transport, explaining the compensation of mitochondrial transport in kif1B knockout mice.

Recent works have shown that the localization of KIF1Bα in mitochondria is controlled by the newly identified KIF1 binding protein (KBP) by yeast two hybrid screens (241). Overexpression of the dominant-negative protein or an antisense construct decreases the activity of KIF1Bα and leads to the aggregation of mitochondria in vivo.

Other authors have reported that seven amino acids in the COOH-terminal region of KIF1Bα selectively interact with the PDZ domains of PSD-95, PSD-97, and S-SCAM, members of a family of synaptic vesicle-associated scaffolding proteins (139). Although their immunocytochemistry data showing a diffuse overlapping distribution of KIF1Bα and these scaffolding proteins in cultured neurons were not sufficiently persuasive, the decrease in the level of KIF1Bα could, to some degree, explain the peripheral neuropathy caused by kif1B mutation.

D. KIF5: A Major Dimeric Motor for Axonal Transport

KIF5, a conventional kinesin, was the first identified and is the most abundant motor protein (68, 230); it has now been revealed to consist of three closely related subtypes: KIF5A, KIF5B, and KIF5C (Fig. 2) (1, 100). While KIF5B is expressed ubiquitously, KIF5A and KIF5C are neuron specific. In contrast to KIF1A and KIF1B, KIF5 proteins form homo- or heterodimers among themselves through the coiled-coil region in their stalk domains (100). Within cells, about half of the KIF5 proteins present are neuron specific. In contrast to KIF1Bα, KIF5A and KIF5C may minimize an overt effect caused by depletion of this molecule.

KIF5 proteins play essential roles in axonal transport. In Drosophila, there is only one kif5 and one kinesin light chain gene. Therefore, Drosophila mutants of kif5 show a severe motor neuronal disease phenotype and lethality (84, 189). Within axons, “organelle jams” stacked with membrane vesicles and organelles were observed, and these obstructions disrupted both anterograde and retrograde transport. The same phenotype is also caused by the loss of KLC in Drosophila (43). On the other hand, in mouse, there exist three KIF5 proteins and at least two KLCs with many spliced variants (100, 131, 176). In spite of existence of multiple KIF5 proteins, disruption of neuron-specific KLC1 results in an aberrant pool of KIF5A in the peripheral portion of the Golgi apparatus (177), suggesting differentiated functions among KIF5/KLC subtypes. When kif5A was conditionally targeted by a synapsin-promoted Cre-recombinase transgene, young mutant mice showed no sign of interrupted transport within axons, but an accumulation of neurofilament in the cell body, suggesting a role for KIF5A as a neurofilament motor (243) (Fig. 4). This could explain the effect of a missense mutation in kif5A in patients with hereditary spastic paraplegia (179). In fact, this mutation disrupts microtubule-activated kinesin activity in vitro and may inhibit the axonal transport of neurofilament in vivo. In contrast to KIF5A, kif5C knockout mice survive with no abnormality except for a reduction in brain size (100). Reflecting the relative abundance of KIF5C in motor neurons, the number of motor neurons was decreased by 28%. As upregulation of KIF5A or KIF5B was not detected in the kif5C knockout brain, the restricted distribution of KIF5C may minimize an overt effect caused by depletion of this molecule.

To investigate the intracellular functions of KIF5 proteins, depletion of these proteins from cell culture systems was performed using antisense oligonucleotides (36). In hippocampal neurons, induction of antisense oligonucleotides against kif5 reduced the overall length of neurites and inhibited the transport of GAP-43 and synapsin to the tips of neurites, showing specific transport of these two molecules by KIF5 proteins. By conventional approaches that disrupt the function of one motor and allow the localization of known transported molecules to be examined, we were able to determine whether each motor is essential for transport of a specific molecule being transported; however, since this approach requires the knowledge of the molecule being transported, we still have no way to find a motor for unknown molecule. New technological progress now makes it possible to identify new binding partners, by the aid of a mass spectrometric approach combined with immunoprecipitation, or with GST pulldown experiments; alternatively, they can be identified directly by using yeast two-hybrid systems.

With the use of these methods, an increasing number of molecules have been reported to be involved in the
transport of cargoes by KIF5 proteins. While synaptic vesicle precursors are conveyed by monomeric motors, such as KIF1A and KIF1Bβ, SNARE proteins, which are essential for the docking of synaptic vesicles at presynaptic membranes, are reported to be carried by KIF5s. Direct binding between SNAP25 and the cargo-binding domain of KIF5 proteins was recognized using a yeast two-hybrid system and confirmed by an in vitro binding assay (29) (Fig. 4). Syntaxin was also found to bind to KIF5 proteins via syntabulin, a new partner that shares homology with the p150 subunit of the dynactin complex (212). Syntabulin and syntaxin transport are suggested to be independent of KLC, since syntabulin binds directly to the COOH-terminal region of KIF5 in an in vitro assay (Fig. 5).

As stated in the previous section, mitochondria are also transported by KIF5 proteins. Mitochondria accumulate in the centers of cells when the ki5B gene is disrupted (215). In mice, a ki5B null mutation is embryonic lethal, but the mitochondrial phenotype in yolk sac-derived cultured cells from ki5B null mice could be rescued by exogenous expression of either KIF5A, KIF5B, or KIF5C, suggesting that any type of KIF5 can transport mitochondria separately (100) (Fig. 4). Some investigators have reported that hyperphosphorylation of KLC inhibits the function of KIF5 proteins and causes clustering of mitochondria in the perinuclear region (25); however, others have shown KLC-independent axonal transport of mitochondria mediated only by milton and KIF5 (44) (Fig. 5). These authors showed a competitive interaction between milton and KLC for KIF5 using cotransfection experiments (44). KLC may function to regulate the motor activity of KIF5, but not be essential for binding mitochondria. Recently, syntabulin was also reported to possess another binding site for mitochondria, but its relationship to milton remains to be determined (19, 44).

Using a combination of pulldown assays and mass spectrometric analysis, the direct binding of DISC1 and KIF5 was recently identified; these molecules then form a cargo complex with NUDEL, LIS1, and 14–3-3ε (217). Although DISC1 has been reported to function with cytoplasmic dynein rather than KIF5, the transport of this complex by KIF5 contributes to axonal elongation in neurons.

Although an increasing number of cargo molecules have been reported, not all components or functions have been revealed yet. In some cases, only binding has been recognized at present. For example, β-dystrobrevin, a dystrophin-related protein, has been recognized to bind directly to KIF5A and KIF5B (127). Other disease-related proteins have also been reported to be associated with KIF5 complex. While neurofibromin binds directly to KIF5, huntingtin-associated protein-1 and torsinA bind indirectly via KLC. Their precise function in vivo is under investigation (52, 98, 132).

Cargo molecules that bind directly to KLC have also been identified. These usually bind KIF5 tetramers via the tetratricopeptide repeat (TPR) domain of KLC (30). Accumulating data have revealed a particular population of cargoes that KIF5s bind indirectly via KLCs. Among them, c-jun NH2-terminal kinase (JNK)-interacting proteins (JIPs) are well characterized (14, 234). In mammals, three subtypes of JIPs (JIP1–3) have been identified (89). The first evidence of a relation between JIPs and KIF5 proteins came from studies of Drosophila. Mutants of the Drosophila homolog of JIP3, Sunday driver (SYD), caused aberrant accumulation of axonal cargoes, closely resembling the phenotype of kinesin mutants. With the use of the yeast two-hybrid method, SYD was found to bind to the TPR domain of KLC (14). JIPs were also identified by yeast two-hybrid analysis and immunoprecipitation during search for a binding partner of KLC (234). JIPs function primarily as scaffolding proteins to mediate the JNK signaling cascade by directly binding to MAPK, MAPKK, and MAPKKK (102). On the other hand, they bind to KLC and connect ApoER2 Reelin receptor-containing vesicles to the motor protein complex (234) (Figs. 4 and 5). The clarification of the relation between two functions of JIPs needs further experiments.

An antibody against amyloid-β precursor protein (APP) immunoprecipitates KIF5B and KLCs. Direct binding of APP to the TPR domain of KLC has also been recognized (95, 97) (Figs. 4 and 5). Although the specificity of binding was confirmed in KLC-1 knockout mice, no direct interaction was detected between APP and KLC in other experimental systems (120). As no change in the transport driven by KIF5 was observed in APP knockout mice, the function of APP as an adapting protein for KIF5 is now controversial. A subsequent finding that linked the two conflicting lines of evidence showed that JIP1 enhances a weak association between APP and KLC by binding simultaneously to APP and KIF5 (146). JIP1 is also reported to accelerate the phosphorylation of APP by JNK, and to assist the transport of phosphorylated forms of APP only (88). These data may explain the limited effect of KIF5 on APP transport.

E. KIF3/Kinesin-II: A Heterodimeric Motor for Axonal Transport

KIF3 proteins are categorized in the kinesin-2 family. They are ubiquitously expressed in tissues and are especially abundant in the brain. They usually exist as a heterotrimeric complex of KIF3A, KIF3B, and kinesin superfamily-associated protein 3 (KAP3) with a stoichiometry of 1:1:1 (1, 62, 67, 111, 237, 246, 247). As KAP3 binds to the tail domains of KIF3s (Fig. 3), KIF3s bind to cargoes through the Armadillo repeats of KAP3. KIF3s steer not only the transport of vesicles, but also intraflagellar rafts.
in cilia and flagella (22). The developmental effect of disruption to KIF3s on ciliogenesis will be discussed in section VIII.A.

In neurons, fodrin has been recognized as a binding partner of KAP3 by yeast two-hybrid experiments. The transport of vesicles containing fodrin by KIF3 proteins is essential for neurite elongation, which is blocked by microinjection of antibodies against KIF3 into cultured superior cervical ganglion neurons (213) (Figs. 4 and 5).

KIF3 proteins are also essential for the polarization of neurons. The tumor suppressor gene adenomatous polyposis coli (APC) binds to KAP3 via an interaction that is mediated by the Armadillo repeats of both molecules (200). Exogenous expression of a partial deletion mutant of KAP3 that binds APC but not KIF3 selectively inhibited the transport of APC to the tips of protrusions, suggesting transport of APC by KIF3 via KAP3. In neurons, APC forms a complex with Par3, and its accumulation at the tip of the axon is essential for the polarization of neurons. A direct interaction between Par3 and the tail domain of KIF3A has also been reported (154) (Fig. 5). Considering that APC also binds to glycogen synthase kinase-3β (GSK-3β), a determinant of the fate of axons that is downstream of phosphatidylinositol 3,4,5-trisphosphate (PIP3), it can be concluded that transport of the APC complex by KIF3 proteins plays a key role in the axonogenesis of neurons.

Furthermore, KIF3 proteins have been shown to transport choline acetyltransferase and acetylcholinesterase in Drosophila axons. Although siRNA-mediated knockout of each component of the motor complex disrupted the transport of both enzymes, these two enzymes when they were labeled fluorescently were found to be transported independently with different properties within the same axon. Different molecular weight splice variants of KIF3A appear to be involved in the transport of these two enzymes (10). A recent paper showed the involvement of KIF3 proteins in the transport of axonal voltage-dependent potassium (Kv) channels (46). Fluorescence resonance energy transfer (FRET) analysis also revealed the cotransport of Kvβ2 and EB1 along the axon. Whether or not the targeting mechanism of these microtubule plus-end-tracking proteins (+TIPS) to the plus end of microtubules contributes to transport of these channels will be revealed by further experiments.

F. KIF13B/GAKIN

KIF13B, another member of the kinesin-3 family, was first identified by an improved PCR strategy (148). Subsequently, it was also analyzed as a guanylate kinase-associated kinesin (GAKIN) by GST pulldown using the guanylate kinase-like (GUK) domain of the discs large tumor suppressor protein (Dlg) (54). In Drosophila neuroblasts, the transport of Dlg by KIF13B induces astral spindles at the apical cortex through the interaction of Dlg with Pins, Par and Insc, which is essential for determining cortical polarity in a microtubule-dependent manner (205).

KIF13B was also found to be a binding partner of centaurin-α1 by yeast two-hybrid experiments (232). Centaurin-α1 is known as a molecule with a double function: as a GTPase-activating protein (GAP) for the ADP-ribosylation factor (ARF), and as a phosphatidylinositol 3,4,5-trisphosphate (PIP3)-binding protein. Interestingly, centaurin-α1 binds specifically to a forhead-associated (FHA) domain in KIF13B, commonly locating in the NH2-terminal stalk domain of the kinesin-3 motor (Fig. 2). KIF13B recruits centaurin-α1 to the plasma membrane of the leading edge and regulates the activity of ARF6 through its GAP function in nonneuronal cells. In neurons, KIF13B is reported to bind PIP3 via centaurin-α1 and transports PIP3-containing vesicles to the tips of axons (Figs. 4 and 5). This process seems to initiate axonal differentiation, as overexpression of KIF13B induces the formation of multiple axon-like neurites that contain abundant PIP3 (79). The role of KIF13B in ARF6 activation in neurons remains to be determined.

IV. DENDRITIC TRANSPORT IN NEURONS

In contrast to axons in which microtubules run unidirectionally, with their plus ends directed to the distal ends, the polarity of microtubules in proximal dendrites is mixed, while in the distal dendrites, the polarity is the same as in axons. Some dendritic motors, such as KIF17, sense the difference between the two types of neurite and exist predominantly in dendrites, while other motors, like KIF5 proteins, transport cargoes in both dendrites and axons.

A. KIF17: An NMDA Receptor Transporter

KIF17, an NH2-terminal motor domain-type motor, is a member of the kinesin-2 family, along with the KIF3 proteins (148, 195). In contrast to KIF3 proteins, KIF17 is mainly localized in the cell bodies and dendrites of neurons. By yeast two-hybrid assay, Lin-10 (mouse homolog of Caenorhabditis elegans LIN-10), also known as Mint1, was identified as a binding partner of KIF17. Mint1 has two PDZ domains, and the KIF17 tail domain binds to the first of these. This interaction was also confirmed by immunoprecipitation using an anti-mLin-10 antibody and a BIAcore system. Via mLin-10, KIF17 was found to bind successively to mLin-2, mLin-7, and finally to the NR2B subunit of N-methyl-D-aspartate (NMDA)-type glutamate receptors, all of which are members of a previously identified complex on NMDA receptor-binding vesicles (93) (Figs. 4 and 5).
An in vitro reconstruction comprising the KIF17-cargo membrane fraction, *Chlamydomonas* flagellum microtubules, KIF17 and ATP, succeeded in moving cargo vesicles to the plus ends (195). These vesicles have also been shown to contain NR2B by immunocytochemistry. These results collectively indicate that KIF17 transports the NMDA receptor subunit NR2B via an interaction with the tripartite scaffolding protein complex containing mLin-10, mLin-2, and mLin-7.

The intracellular function of KIF17 was investigated using a fluorescence-tagged expression system in cultured neurons. YFP-KIF17 is distributed mainly in the cell body and dendrites, but not in axons. As YFP-KIF17 colocalized with NR2B, but not with PSD-95, in postsynaptic region, it was supposed that it was being transported (47). Functional blocking of KIF17 by the expression of antisense oligonucleotides or using dominant-negative mutants resulted in a significant decrease in the density of NMDA receptor clusters. When the NMDA receptor was functionally blocked by the antagonist AP-V, the expression of KIF17 as well as that of NR2B subunits was upregulated, suggesting the coregulation of KIF17 and NR2B at the transcriptional level.

The in vivo role of KIF17 was examined in a transgenic mouse overexpressing KIF17, using the calmodulin-dependent kinase II (CaMKII) promoter (239). The mice showed significantly better performance in behavioral tests, such as the Morris water maze tasks for working memory and spatial memory, suggesting better learning and memory than wild-type mice. In addition to the increase in the levels of KIF17 and NR2B proteins in the hippocampus and cerebral cortex, the mRNA expression levels of both molecules were also high. The upregulation of NR2B and KIF17 transcription was confirmed by an increase in the amount of phosphorylated cAMP-response element-binding protein (CREB). A CREB consensus sequence was found in the promoter regions of both genes. The enhanced dendritic transport of the NR2B subunit may stimulate the transcription of NR2B subunit and KIF17, enhance synaptic transmission, and ultimately induce an improvement in learning and memory in transgenic mice, showing that motor proteins play significant roles in higher-order brain functions.

B. KIF5: An AMPA Receptor Transporter

As noted in the previous section, KIF5 proteins play a major role in fast axonal transport. Moreover, a role in dendritic transport has also been identified for KIF5 proteins. Yeast two-hybrid assays using the cargo-binding domain of KIF5 as bait identified glutamate receptor-interacting protein 1 (GRIPI) as a potential binding partner. GRIPI is known to bind to the GluR2 subunit of α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)-type receptors, another type of glutamate receptor in dendrites (196). Immunoprecipitation experiments showed that GRIP1 bound to KIF5 and the GluR2 subunit in neurons (Figs. 4 and 5). In addition, when a KIF5 dominant-negative construct lacking the motor domain was expressed in cultured neurons, the density of GluR2 clusters in dendrites significantly decreased, suggesting that GluR2 is normally transported to dendrites by KIF5 via its interaction with GRIP1.

Furthermore, when the minimal KIF5-binding domain of GRIP1 was expressed in hippocampal neurons, KIF5 proteins were recruited to dendrites, while the expression of JIP-3, a well-known binding protein for KLC (14, 234), led KIF5 proteins to localize predominantly in axons. These results suggest that the binding of GRIP1 to KIF5 tends to steer the motor towards dendrites rather than axons, although the mechanism has yet to be elucidated.

C. KIF5: An mRNA Transporter

GST pulldown assays using the tail domain of KIF5 proteins isolated large RNase-sensitive granules containing hnRNP-U, Pur-α, and Pur-β, all of which have been identified as members of an mRNA/protein (mRNP) complex released from ribosomes following EDTA treatment (99, 163). This finding suggested that the mRNP complex is transported by KIF5 proteins (Fig. 4). Further experiments identified various RNA-associated proteins, such as PSF, DDX1, DDX3, SYNCRIP, FMRPs, and staufen, which were consistently coimmunoprecipitated by antibodies against RNA-associated proteins and KIF5 (99). Specific mRNAs, such as those for CAMKIIα and activity-regulated cytoskeleton-associated protein (Arc), but not that for tubulin, were detected in the same granules, suggesting the transport of specific mRNAs by KIF5 proteins. Real-time movement of Pur-α-containing granules in the dendrites of cultured neurons was suppressed by RNA interference (RNAi) of hnRNP-U, Pur-α, PSF, or staufen, but not by RNAi of DDX3 or SYNCRIP, suggesting the existence of an essential subpopulation of members of granules for the transport of mRNAs. Proteomic analysis of these granules has identified at least 42 associated proteins to date. A close examination of interactions among these proteins will clarify the regulation mechanism of RNA transport.

The RNP granules were further examined by labeling different marker proteins. Compatible with the bidirectional movement of granules, cytoplasmic dynein was detected in the immunoprecipitated fraction, but KLC was not; this finding is compatible with the fact that the knockdown of KLC did not affect RNP complex transport (125).
D. KIFC2

KIFC2, a member of the C-kinesin or kinesin-14 family, is abundantly expressed in the adult brain (Fig. 2) (57, 148, 185). Although other major C-kinesins, represented by Kar3 and Ncd, are implicated in cell division, KIFC2 is supposed to function in membrane transport in postmitotic neurons (185). Immunofluorescence data showed that KIFC2 was localized to punctate structures in cell bodies and dendrites. Immunoprecipitation experiments isolated multivesicular body (mvb)-like organelles, suggesting that KIFC2 is a motor for mvb-like organelles in dendrites.

E. CHO1/MKLP1

CHO1, or MKLP1, is a member of the kinesin-6 family (Fig. 2). CHO1 was first identified as a mitotic motor (194). By transporting the minus end of microtubules towards the plus end of other microtubules, CHO1 transports microtubules of opposite orientation toward one another, a process important for spindle elongation during anaphase. During interphase, CHO1 was found to exist in the dendrites of neurons. Expression of antisense oligonucleotides against CHO1 decreased the population of microtubules with their minus ends at distal sites in neurites, making the appearance of neurites more axonlike (199). Whether mixed directionality of microtubules in dendrites is performed by CHO1 or not will be investigated in the future.

F. KIF21B

KIF21B, a member of the kinesin-4 family, is a plus-end-directed motor, highly enriched in dendrites (Fig. 2). KIF21B has a cluster of negatively charged amino acids within its stalk domain and seven WD-40 repeats within its tail domain (128), which is supposed to bind cargoes. Further characterization of the function of KIF21B is necessary.

V. CONVENTIONAL TRANSPORT, INCLUDING ENDOPLASMIC RETICULUM TO GOLGI, LYSOSOMES, AND ENDOSOMES

Within cell bodies, various membrane organelles communicate with one another through vesicular transport. To maintain this communication, the recruitment and integration of membranes to the proper cytoskeletons are essential, processes that are strictly supported by microtubule- and actin-dependent motor proteins.

A. Transport Between the Endoplasmic Reticulum and Golgi Apparatus

Vesicular structures are budded from the endoplasmic reticulum (ER), which is reticularly distributed throughout the cell body, and transported towards the Golgi apparatus, which is concentrated in the perinuclear region in mammalian cells. This transport is bidirectional, and the forward transport that is minus-end-directed is implicated by cytoplasmic dynein. Reverse transport, from the Golgi to the ER, can be visualized by treatment with brefeldin A, which only blocks ER-to-Golgi transport (138). In 1992, kinectin was identified as a binding partner of KIF5 proteins (225). Although an antibody against kinectin was at first reported to inhibit axonal transport, isoforms of kinectin expressed in neurons were revealed to have no specific binding domain for KIF5. The variable COOH-terminal region of kinectin that binds directly to the tail region of KIF5 inhibits the microtubule-activated ATPase activity of KIF5 (169). Thus kinectin may function as an inhibitor of the transport by KIF5 in nonneuronal cells.

The major isoform of kinectin, with a molecular mass of 160 kDa, is concentrated in the ER of nonneuronal cells and is supposed to function in ER extension (187). However, KIF5 and kinectin seem to be dispensable for ER extension, since ER structure remains normal in kif5B or kinectin knockout mice (174, 215). In normal rat kidney cells, microinjection of an antibody against KIF5 (H1 monoclonal antibody) blocked Golgi-to-ER transport, but not ER-to-Golgi transport, suggesting that KIF5 is involved in backwards, recycling transport (126). Recent work with KLCs has characterized the different KLC isoforms bound to purified membrane fractions originating from the ER and Golgi apparatus (240) (Fig. 4). Indeed, preincubation of these fractions with GST-fused cargo-binding domains of KLC1B and 1D blocked the in vitro motility of the ER and Golgi fractions, respectively. In vivo and in vitro assays showed that KIF5B steers ~50% of the transport between these organelles and tends to bind tubules extended from Golgi following brefeldin A treatment. Colocalization of KIF5 with organelle-marker proteins implicated KIF5 in the transport of a substantial population of cargoes that contained ERGIC58 and p115 but not KDEL receptor or COPs (240).

KIF1C, a member of the kinesin-3 family, is also reported to participate in this transport (Fig. 2). Under brefeldin A treatment, the inactive form of KIF1C blocked Golgi-to-ER transport (32). Disrupted Golgi-to-ER transport was reported neither in kif1C nor in kif5B knockout mice, suggesting a redundant function of the two motors in this transport (149, 215) (Fig. 4).

In Xenopus cell lines, Xklp3s, Xenopus homologs of KIF3 proteins are colocalized with KDEL receptor that is recycling between the ER and Golgi; thus KIF3s are sup-
posed to be the third motor involved in this transport, although discrepant behavior of marker proteins suggested the existence of multiple cargoes (Fig. 4). KAP3 silencing using an RNAi approach resulted in the fragmentation of the Golgi apparatus and changed the localization of KDEL receptor, revealing a role for KIF3 proteins in KDEL receptor-dependent Golgi-to-ER transport (209).

Besides transport, some motor proteins have been recognized to sustain perinuclear positioning of the Golgi apparatus. Rabkinexin-6, which was identified as a specific binding partner of the GDP-bound form of Rab-6, induced the scattering of the Golgi apparatus to the periphery of cells when overexpressed (33) (Fig. 2). When cytoplasmic dynein or kifc3, a member of the C-kinesin family, is targeted in mouse, depletion of these minus-end-directed motors also affects the positioning of the Golgi, suggesting a tug-of-war between these three motors for the integrity and position of the Golgi apparatus (59, 244) (Fig. 2).

B. Lysosomal Transport

Lysosomes are dynamic organelles that move centrally or peripherally, according to a decrease or increase, respectively, in the pH of the culture medium (63). The dynamic dispersion of lysosomes during the recovery phase of subsequent acidification, is blocked by exogenous expression of rigor KIF5 (T93N), a mutant KIF5 that has no ATPase activity and rigorously attaches to microtubules (151). The same inhibitory effect was also observed in kif5B knockout cells, or in cells expressing polypeptides corresponding to the kinesin binding domain of kinectin (169, 215) (Fig. 4).

Melanosomes are originally lysosome-related organelles and move dynamically within the cells. Competition between one minus-end-directed motor, cytoplasmic dynein, and two plus-end-directed motors, KIF5B and KIF3 (kinesin-II), along microtubules, regulates their movement (45). Because melanosomes arriving at the cell periphery are tethered to actin filaments by melanophilin, myosin Va and Rab-27, the effect of plus-end-directed motors is only visible after perturbation of central aggregation with melatonin, followed by treatment of cells with MSH, which stimulates dispersion (82, 175, 242). In mammalian melanocytes, antisense oligonucleotides against KIF5B promote perinuclear aggregation, suggesting an essential role for KIF5B in pigment dispersion (58). However, in Xenopus cells, the dominant-negative form of Xklp3, the Xenopus homolog of KIF3, but not a blocking antibody against KIF5B, inhibits pigment dispersion (227), which may reflect a difference in functional motors among species (Fig. 4).

C. Transport From the Trans-Golgi Network to the Plasma Membrane

Transport from the trans-Golgi network (TGN) to the plasma membrane is often compared with dendritic transport in neurons, because in some polarized nonneuronal cells there is another specific type of transport, similar to axonal transport in neurons. The default pathway is called basolateral transport, and the alternative is apical transport. Vesicular stomatitis virus glycoprotein (VSV-G) and influenza virus hemagglutinin (HA) are representative viral markers of the default and novel pathways, respectively.

KIF13A, a member of the kinesin-3 family, exists as a dimer in vivo, although recombinant KIF13A expressed in Escherichia coli was purified as a monomer (147, 148). A GST pulldown assay using the tail domain of KIF13A recognized an ear domain of β1-adaptin, a subunit of the AP-1 complex that is engaged in vesicular transport from the TGN to the plasma membrane. γ-Adaptin and the mannose-6-phosphate (M6P) receptor were also coprecipitated from the Triton-X-100-solubilized membrane fraction using an anti-KIF13A antibody, suggesting a detergent-resistant interaction among them (Figs. 4 and 5). Overexpression of KIF13A resulted in a redistribution of the M6P receptor to the cell periphery, suggesting a role for KIF13A as a transporter of M6P receptor-containing vesicles via the AP-1 complex.

Besides KIF13A, KIF5 is also recognized as a motor for transport towards the plasma membrane. Previous work has shown, however, that colocalization of KIF5 and VSV-G is observed only transiently, just at the level of the Golgi, shortly after removal of the perturbation that pools VSV-G at the ER (126). However, recent reports have shown a commitment of KIF5 in this transport. Vaccinia virus A36R membrane protein is reported to bind directly to the TPR region of KLC and be transported to the plasma membrane (181). Substrate adhesions in Xenopus fibroblasts also require KIF5 for keeping their size and number at the plasma membrane, a process that was blocked by microinjection of an anti-KIF5 antibody (SUK-4) or by the expression of a motorless construct (116). In either case, there was no evidence for where exactly these cargoes originated from, but the TGN is the most probable source. The cell body itself is so packed that it is sometimes difficult to discriminate transport from the TGN to the plasma membrane, from endosomal recycling, which is referred to in the next section.

In polarized epithelial cells, an apical transport develops in addition to the default transport starting from the TGN. In polarized MDCK cells, KIFC3, a member of the C-kinesin family, transports TGN-derived vesicles containing annexin XIIb and HA to the apical plasma membrane, possibly in cooperation with cytoplasmic dynein (158).
D. Endosomal Recycling

Vesicular structures recycle between subcellular compartments and the plasma membrane. They are at first endocytosed from the plasma membrane as early endosomes. Through fission and fusion, they develop into late endosomes and then into lysosomes for degeneration, or to recycling endosomes for recurrence at the plasma membrane through exocytosis. The small GTPases of the Rab family are known to regulate the sorting of these membranes through locating different members at different compartments (94). Some Rab proteins have been revealed to control the association of specific motor proteins with particular endosomes.

Multiple motor proteins have been identified on endosomes (Fig. 4). A tug-of-war between motors driving cargoes in opposite directions determines the localization of membranes (7). Which motor proteins are implicated and regulated by a certain Rab protein seems to depend on the cell type. In 3T3-L1 adipocytes, early endosomes containing Rab-5 were reported to be endocytosed by cytoplasmic dynein, and replacement of Rab-5 with Rab-4 induced KIF3-mediated exocytosis of the endocytosed vesicles (86); both of these transports by cytoplasmic dynein and KIF3 were triggered by insulin. Contrarily, when endosomes are purified from the liver, early endosomes labeled with Rab-4 are driven by KIF5B and KIFC2. The GDP-bound form of Rab-4 recruits KIFC2 to the membrane, which keeps endosomes away from the plasma membrane. When they are transformed to late endosomes, these vesicles are labeled with Rab-7 accompanied by cytoplasmic dynein and KIF3A, and sometimes by KIFC2 (8, 9). The KIF3 complex is also detected on late endosomes and lysosomes in Hela and COS-7 cells (17). RNAs of KAP3, which binds cargoes to KIF3 tail domain, or expression of motorless KIF3A, changed the distribution of late endosomes and lysosomes. However, in spite of this altered distribution, their function, the uptake and delivery of receptors and ligands through fission and fusion, was not affected by the absence of motor proteins, suggesting that, with regards to endosomes, the function of motor proteins just limits the movement of vesicles, and that the transformation of vesicles rather depends on Rab proteins.

KIF16B, a member of the kinesin-3 family (148), binds PIP$_3$-containing vesicles via the PX domain in its tail region (Figs. 2, 4, and 5). In vivo, KIF16B was localized to PIP$_3$-positive early endosomes, and this association was blocked by the persistent GTP-bound form of Rab-5 and the specific inhibitor of phosphorylcholine-3-OH kinase vHPS34 (77). Plus-end-directed motility of KIF16B fixed endocytosed epidermal growth factors (EGFs) and EGF receptors beneath the plasma membrane and prevented them from entering the degenerative pathway. This sustaining function of KIF16B is in contrast to that of KIF3, which is to enhance the exocytosis of cargoes. Another member of the kinesin-3 family, KIF1C, is also localized at podosomes at the plus end of microtubules in the peripheral regions of macrophages, in addition to their distribution in the Golgi region at the centers of cells (113).

In close proximity to the plasma membrane, microtubule motors share vesicle transport mechanisms with members of the myosin motor family, which move along the actin network. Among KIFs, there exists a novel kinesin-1 member in *Dictyostelium discoideum* that was reported to have an actin-binding region in its tail domain. The exact function of this region remains to be seen (90).

VI. SLOW AXONAL TRANSPORT

In addition to the fast axonal transport referred to in previous sections, there exists slow axonal transport in neurons, which carries cytoskeletal proteins, such as tubulins and neurofilament proteins and glycoytic enzymes at a velocity of 0.1–3 mm/day (219). This form of intracellular transport is reported to be driven by the microtubule-dependent motor proteins KIF5 (220). Regarding the size of cargoes transporting cytoskeletal proteins, there has long been a debate between the “oligomer (complexes of several molecules) hypothesis” and the “polymer (filaments) hypothesis” (16, 197, 220, 245). The majority of discrepancies have arisen from the differences in the methods used. Some researchers overexpressed fluorescently labeled proteins in culture systems to investigate their movement and insist that the fast but infrequent movement of polymers by fast axonal motors causes only a small displacement of molecules (21, 235, 236, 248). However, it remains debatable whether they have reproduced in their culture system slow axonal transport observed in vivo.

On the other hand, others have observed the slow movement of injected proteins in squid giant axons and estimated the speed of movement, diffusion coefficients or diffusion times during displacement, from fluorescence profiles (39, 40, 220). These investigators used confocal laser scanning microscopy or fluorescent correlation microscopy and showed that there is a significant difference in diffusivity characteristics between soluble protein and tubulin, between tubulin and taxol-stabilized polymerized tubulin, and between tubulin and neurofilament. In another study, the transport of injected oligomers of neurofilament-M was observed in neurofilament-depleted axons derived from transgenic mice with neurofilament-H-$\beta$-galactosidase that cannot form filament with neurofilament-M and -L (221). Thus these data propose transport of neurofilament in the form of oligomers. Collectively, from these findings, tubulin is observed to be constantly transported in the form of oligomers (40, 197, 220, 221). This conclusion seems to be persuasive at present. However,
how cytoskeletal molecules are transported by KIF5 remains unknown. Recently, KIF5 was reported to transport tubulin dimer via collapsing response mediator protein-2 (crmp-2) and KLC (107) (Figs. 4 and 5). The relation of the identified transport mechanism to slow axonal transport was not discussed and needs further examination with regard to dynamics.

VII. POLARIZED SORTING BY MOTOR PROTEINS

After molecules arrive at the targeted membrane, they are specifically incorporated into that membrane in one of two ways: they are either selectively fused into the membrane, or they are indiscriminately captured and remain on the membrane by selectively escaping endocytosis (152, 186). Many interactive sequences for specific recognition have been identified as sorting signals in the cytosolic domains of targeted molecules (70). Additionally, motor proteins appear to engage in polarized sorting, presumed from the fact that exogenous expression of truncated motor domains resulted in their polarized localization, although this could not be influenced by the cargoes usually bound to the cargo-binding domain in the COOH-terminal portion of these motor proteins (91, 150). Thus selective binding to a particular motor limits the possible destination, because some motor proteins are restricted to enter either axons or dendrites.

To elucidate the molecular mechanism underlying polarized sorting by motor proteins, the distribution of the rigor mutant motor protein was investigated following infection of differentiated cultured hippocampal neurons using an adenovirus vector (150). Because the rigor mutant, which lacks ATPase activity, does not move along microtubules once bound, it remains localized on the microtubules where it was first recruited. While tailless KIF5 tended to accumulate at the tip of axon, rigor-KIF5B (G234A) distributed to a restricted portion of microtubules localized between the cell center and the initial segment of the axon. The initial segment is known to work as a diffusion barrier to inhibit backwards leak of membrane proteins once targeted to the axon (238). Thus selective binding of KIF5 to microtubules in the initial segment enables the transport of specific proteins into the axon. Treatment with low doses of taxol inhibited this selectivity, suggesting the importance of microtubule dynamics of the initial segment for the polarized sorting mechanism. In contrast to KIF5, rigor KIF17 was localized to the initial segment as well as dendrites, reflecting the distribution of tailless KIF17 in both axons and dendrites (150).

Interactions between motor proteins and specifically modified tubulins have been suggested to underlie another polarized sorting mechanism. Some tubulins are posttranslationally modified within their COOH-terminal domains, and incorporated into the characteristic microtubules as acetylated, detyrosinated, glycylated, or glutaminated forms. Indeed, perturbation of acetylation and glutamination affected the transport of KIF5 and KIF1A, respectively (31, 85, 178).

Observation of neurons during development has also clarified the existence of multiple mechanisms for axonal targeting. Even in premature neurons only 2 days in culture, tailless KIF5 selectively accumulated at the tip of a single neurite (91). However, tailless KIF1A, another axonal transport motor distributed at the tips of all neurites at this stage, suggested that another sorting mechanism targets KIF1A to the axon, which is established after the complete polarization of neurons.

VIII. DEVELOPMENT AND MOLECULAR MOTORS

To investigate the functions of motor proteins at the tissue and individual animal levels, molecular genetic approaches have been attempted and have shown that intracellular transport by motor proteins and some motor proteins with unique functions are critical for many aspects of functions at the tissue and animal levels.

A. KIF3: Left-Right Determination and Development

In addition to their involvement in axonal transport and conventional transport, KIF3 heterodimers have a specific function in intraflagellar transport (IFT). IFT was first reported as the bidirectional movement of two granulelike particles beneath the flagellar membrane, which is essential for the assembly of cilia (115). These two particles, A and B, bind two kinds of kinesin-2 motors with different velocities, kinesin-II (KIF3s, FLA10) and Osm-3, respectively, in addition to retrograde motors, flagellar dyneins, resulting in an unusual type of transport in which particle complexes are conveyed at an intermediate velocity between those of kinesin-II and Osm-3 (170). kif3A and kif3B knockout mice showed very similar phenotypes (161, 214). A lack of KIF3A or KIF3B in mice is embryonic lethal, and embryos on 11.5 dpc show various anomalies, including an abnormal cardiac looping with equal frequency of L-loop and D-loop, which implies randomization of left-right determination (161, 214) (Fig. 7).

At the molecular level, left-right determination can be detected by the unilateral distribution of marker molecules: a triangular ventral dent in early embryos, called the “node,” is known to regulate the expression of this series of genes specifically on the left side. Whole-mount in situ hybridization of the nodes of kif3B knockout mice
revealed that the expression of lefty2, the most upstream gene in the pathway regulating left-right determination, was already randomized. Scanning electron microscopy of nodal cells revealed the absence of monocilia in the nodes of kif3B knockout mice (Fig. 7). Immunocytochemistry showed the localization of KIF3A/KIF3B in the monocilia in the wild-type nodes (161, 214). Considering also that the Clamydomonas KIF3 homolog FLA10 is implicated in IFT of the ciliary components from the base to the tips of cilia along microtubules (22, 67), it is supposed that cilia are not formed in kif3B knockout mice because of the absence of KIF3.

To clarify the mechanism underlying the disruption to left-right asymmetry in kif3B knockout mice, further work was attempted. When nodal cilia of wild-type mice, which were thought to be immotile, were observed by video microscopy, the cilia were unexpectedly rotating clockwise at ~600 cycles/min. Moreover, fluorescent dye-labeled beads added to the extraembryonic fluid in the node moved from the right to the left. A constant leftward flow of extraembryonic fluid in the node, named “nodal flow,” was absent in the nodes of kif3B knockout mice. These data suggest the possibility that the leftward nodal flow contributes to the generation of a concentration gradient of a putative morphogen X towards the left side of the node for left-right determination.

Leftward nodal flow is observed in other vertebrates such as rabbits and medaka fish. A video system with a high temporal resolution enabled a more detailed analysis of ciliary movements with their axes tilted at an angle of 40° posteriorly (167). Because of this tilt, only a leftward, but not a rightward, rotation of the cilium would effectively generate hydrodynamic power in a nearly vertical plane, thereby producing leftward nodal flow.

Membrane parcels labeled with the lipophilic fluorescent dye DiI were observed by confocal microscopy in the nodes of living embryos; these parcels were released from the cell surface and the protruding microvilli of the cell surface, moved rapidly down the stream of the nodal flow, and were finally fragmented at the location close to the left wall (216). This transport was suppressed by the suppression of fibroblast growth factor (FGF) using a specific inhibitor of the FGF receptor tyrosine kinase, SU5402, or a dominant-negative recombinant peptide of an extracellular domain of mouse FGF receptor (FGFR-DN). These parcels, named “nodal vesicular parcels” (NVPs), typically consist of multiple lipophilic granules and have been found by immunostaining to be associated with the downstream morphogen candidates, Sonic hedgehog (SHH) and retinoic acid (RA). These data, originating from research on KIF3 proteins, provide direct evidence that following an FGF trigger, nodal flow transports NVP-associated morphogens including SHH and RA toward the left: this is probably a critical phenomenon of symmetry breaking in mammalian embryos (72).

B. Transport of N-Cadherin in Developing Neurons

Conditional knockout of the kap3 gene, encoding an accessory component of the KIF3 heterotrimer complex that links the KIF3 tail with the cargo, in mice resulted in a nonuniform decrease in the expression of KAP3 in the brain (218). In the cerebral cortex, abnormally hypertrophic regions resembling tumors were distributed in dots corresponding well to the areas lacking KAP3 expression (Fig. 7). In these areas, the levels of N-cadherin and β-catenin at the cell periphery were markedly decreased. In an immortalized embryonic fibroblast cell line with a kap3-null genotype, the release of N-cadherin-GFP from the Golgi apparatus was significantly impaired, leading to a decrease in the amount of N-cadherin-GFP at the cell-cell boundaries. Indeed, the transport of individual post-Golgi organelles containing N-cadherin-GFP showed winding and unstable outward movements in kap3-null cells when observed by time-lapse critical angle fluorescence microscopy. These data showed the KIF3 complex to be a transporter of adhesion molecules such as N-cadherin and β-catenin. β-Catenin is known to have dual functions. When β-catenin is incorporated into adhesion junctions, it contributes to cell-cell adhesion, whereas if it exists in the cytoplasm it tends to get into the nucleus and act as a transcriptional factor with T-cell factor to enhance cell proliferation, thus causing cancer. Thus KIF3 drives the plasma membrane localization of β-catenin, thereby decreasing the levels of β-catenin in the cytoplasm and suppressing tumorigenesis (Fig. 7).

The KIF3 complex was also reported to associate with APC via KAP3 (92). APC is a known tumor suppressor gene that is involved in the degeneration of β-catenin along with GSK-3β and axin. Transport of APC by the KIF3 motor complex is also supposed to contribute to the suppression of tumorigenesis.

C. KIF2: A Suppressor of Collateral Branch Formation

KIF2 is categorized into the kinesin-13 family, members of which have the motor domain in the middle of the molecule; that is, they are M-kinesins. These kinesins consist of three separate subfamilies: KIF2A, KIF2B, and KIF2C/MCAK (Fig. 3). Rather than steering along microtubules with cargoes, KIF2 molecules work as a microtubule depolymerizer. KIF2 steers along microtubules by a diffusion mechanism to the end of microtubules. Then, KIF2 decouples tubulin dimers consecutively from the ends of microtubule filaments (28, 60, 83).

In contrast to the KIF2C subgroup, as represented by MCAK which functions in mitotic stages (28), KIF2A is expressed predominantly in the juvenile brain, especially in neuronal growth cones (159). Kif2A knockout mice...
were born alive, but all died within 1 day (78). Their brains showed laminar defects in the cortex due to the delayed migration of cortical neurons (Fig. 7). Visualization of axon bundles with DiI crystals revealed an increased number of horizontally running neurites in mutant mice, whereas axons run longitudinally in wild-type mice. A more detailed observation of cultured neurons revealed a significant increase in the lengths of axonal collateral branches in the knockout mouse, which consequently hindered the forward migration of the cell body (Fig. 7).

Like KIF2C, KIF2A depolymerizes microtubules in an ATP-dependent manner in vitro (28, 78). When the behavior of fluorescently labeled microtubules was observed in the periphery of wild-type cells, elongating microtubules usually began to depolymerize when reaching the cell periphery. In contrast, in the absence of KIF2A, microtubules continued to elongate even after reaching the cell periphery. These data collectively showed that KIF2A is important for controlling brain wiring by suppressing excessive elongation of branches as a microtubule depolymerizer, which in turn is essential for the migration of neurons.

Recent work searching for the functional complex containing KIF2A has revealed that KIF2A directly interacts with phosphatidylinositol 4-phosphate 5-kinase β (PIP5Kβ), which is implicated in the endocytosis of the plasma membrane through the production of PIP₂ (157). In addition to the coaccumulation of these two proteins at the tips of developing neurites in vivo, PIP5Kβ was shown to increase the microtubule depolymerizing activity of KIF2A in in vitro assays. The cooperative function of PIP5Kβ and KIF2A was proposed to be a fundamental mechanism for the proper elongation of neurites through the coupling of actin and microtubule networks.

D. KIF4: A Regulator of Neuronal Survival

KIF4 is strongly expressed in juvenile neurons where it localizes in nuclei as well as in growth cones (193). Several homologs of KIF4, categorized into the kinesin-4 family, have been implicated in chromosome segregation during the mitotic phase (130). Indeed, in the mitotic stage, KIF4 was localized to mitotic spindles. Suppression of KIF4 in neurons using antisense oligonucleotides resulted in the accumulation of L1 within the cell body, instead of within growth cones, suggesting a role for KIF4 as a transporter of this adhesion molecule for axonal elongation in neurons (173).

A gene-targeting strategy was applied for the kif4 gene and produced ES cell clones with no KIF4 expression (134). Because kif4 is located on the X chromosome, single homologous recombination is sufficient to generate kif4-null cells. KIF4-null neurons that were differentiated in vitro from the ES cells showed an extraordinarily high survival rate and were resistant to apoptosis (Fig. 7). GST pulldown with the tail domain of KIF4 isolated poly-ADP ribose polymerase-1 (PARP-1) from juvenile mouse brain. Recombinant KIF4 bound directly to PARP-1 and suppressed its enzymatic activity. Under stable conditions, the KIF4 and PARP-1 complex was localized in the nucleus. After the membrane was depolarized by high potassium treatment or electrical stimulation, KIF4 was phosphorylated by CaMKII α dissociated from PARP-1 and released into the cytoplasm, which activated PARP-1 to prevent cell death. In the developing brain, KIF4 by forming a complex with PARP-1 prevents the protective action of PARP-1 on cell death (Fig. 7).

IX. REGULATION OF CARGO BINDING

A. Use of an Adaptor/Scaffolding Protein Complex for Cargo Binding

To transport exact cargoes to the exact destination, a motor has to discriminate the specific cargo at the starting point for binding. For that purpose, cargoes usually present specific adaptors to their motor proteins. As referred to in the previous sections, KIF13A binds to the AP-1 adaptor protein complex, which then recognizes the mannose-6-phosphate receptor, enabling its transport from the TGN to the plasma membrane (Fig. 5) (147). KIF17 binds to mLin-10, and via this adaptor, it binds to mLin-2 and the mLin-7 scaffolding protein complex, and finally to the NMDA receptor NR2B subunit (Fig. 5) (195). KIF5 binds directly to GRIP1, and via GRIP1, to the GluR2 subunit of the AMPA receptor (Fig. 5) (196). Therefore, the tail regions of motor molecules recognize and bind to the adaptor or scaffolding proteins to transport membrane organelles that contain functional membrane proteins. The use of adaptor and scaffolding proteins appears to be one of the basic mechanisms for the recognition and transport of cargoes (70, 71, 110).

In addition to adaptor proteins, lipids also contribute to the specificity of binding. KIF1A and KIF16B specifically bind to phosphatidylinositides, such as PIP₂ and PIP₃, respectively, through the PH domains in their tail regions (Fig. 5) (77, 109). KIF13B binds indirectly to PIP₃ via the PH domain of α-centaurin (232). However, it remains unknown if this specific binding to the lipid contributes to the selectivity of cargoes or just provides a reinforcement of their affinity. In fact, in the case of KIF1A/1Bβ, Rab-mediated control of the loading/unloading of the cargo was recently reported (156). The authors of this study showed that DEN/MADD, a Rab-3GEF (guanine nucleotide exchange factor) directly bound the stalk domain of the motor and was implicated in the loading of Rab-3 with synaptic vesicle precursors by producing GTP-
rab3 from the GDP-form, which started the transport and GTP-Rab-3, in turn, was supposed to be released by GTPase activating protein (GAP) at the vesicle’s destination, the synaptic terminals. Considering that KIF16B was also reported to be displaced from the cargo in the presence of dominant-negative Rab-5 (77), these data may presume a new regulation system for lipid-motor interactions.

Some adaptor proteins are supposed to change the processivity of bound motor proteins along microtubules. Calsyntenin-1 was shown to bind directly to KLC by a yeast two-hybrid system and immunoprecipitation (112). A point mutation in the binding site for KLC did not change the speed of transported vesicles, but instead, tended to increase the cargo fraction that is static or moves retrogradely. Drosophila enabled was also reported to bind KIF5 directly and reduce its transport activity under the control of Abl tyrosine kinase (129).

The precise molecular mechanism involved will be clarified by the further experiments.

B. Autoinhibition/Phosphorylation

When KIF5s exist in the cytoplasm and do not bind to cargoes, they are in a folded conformation (38, 51, 68, 87, 211). Two flexible domains in the stalk region enable an interaction between the tail region and the neck/hinge region localized near the motor domain. For this folding to occur, the presence of five COOH-terminal amino acids is critical to keep KIF5 in the inhibitory folded state; that is, in a low binding state for microtubules or in a low ATPase activity state, in which KIF5 is not activated by microtubules (23, 108). Other reports have also shown the critical role of other tail globular regions for folding (252). To induce the folded conformation, KLC is also essential (233). Autoinhibition is overcome by the binding of cargo to KIF5s.

In the case of KIF1A, which exists as a monomer in the cytosol, an intramolecular interaction between the FHA domain and the following coiled-coil domain was reported to inhibit microtubule binding activity as well as multimerization (121). Disruption of this interaction by point mutation, deletion, or hinge-removing mutation enhanced the transport and accumulation of KIF1A at the distal region of neurites. An autoinhibition mechanism involving the tail and hinge domains has also been reported for Osm-3, a KIF3 homolog (87), and for MCAK/KIF2C (140).

Recent work has revealed, however, that autoinhibition is not necessarily overcome by binding to cargoes (240). The authors of this work showed that additional binding of deleted KLC fragment containing only KIF5-binding domain to the cargo-bound KIF5 complex could inhibit the motility of KIF5 motors. They denied the possibility that newly formed unbound KIF5 complexes in the cytoplasm were involved in this inhibition, because depletion of a motor complex in the cytoplasm did not affect the results. As KIF5 forms a dimer with two binding sites for KLC and the tail domains, an autoinhibition mechanism may doubly regulate the activity of KIF5 proteins. A recent report proposed a dual mechanism for autoinhibition, in which the cooperative binding of FEZ1 and JIP1 to the tail globular domains of KIF5 and KLC, respectively, released the KIF5 complex from autoinhibition (12). FEZ1/UNC-76 binds directly to the tail globular domain, which is in the COOH terminus of the light chain-binding and the cargo-binding domains of KIF5. Different from other cargo molecules, FEZ1 does not accumulate in the axon, when KIF5 is depleted and traffic jams of untransported cargoes form in C. elegans (42). Considering that this molecule is phosphorylated downstream of a specific kinase, it may function as a regulator of the motor protein rather than the cargo adaptor.

Arriving at the destination, cargoes must be released from the motor proteins before being introduced into the targeted membrane or any other destination. Previous work showed that the phosphorylation of KIF5 regulates the binding affinity of cargo vesicles (122, 188). Recently the precise mechanism was found as follows: phosphorylation of KLC by GSK-3β detaches KIF5 from transported cargoes without changing the ATPase activity or microtubule affinity of KIF5 (142, 143). Moreover, recent work with KIF17 clarified that the CAMKII-dependent phosphorylation of KIF17 controlled the interaction between KIF17 and its direct binding partner Mint1/mLIN-10 in vitro as well as in vivo (48). The authors of this study identified the phosphorylated Ser, Ser-1029, at the COOH-terminal tail domain of KIF17, and visualized the phosphorylation-dependent transport and detachment of the cargo by immunocytochemistry and FRET, respectively.

Contrarily, the affinity for microtubules or the motility of KIF5 proteins is reported to be regulated by the phosphorylation of binding proteins. FRET analysis detected the dissociation of KIF5β from β-tubulin III following the phosphorylation of JNK proteins within the axon (208). Collectively, phosphorylations of the motor complex and adaptor protein seem to regulate the unloading and transport efficiencies of the cargo, respectively. However, more data are needed to generalize this rule. In the case of the mitotic motor MCAK, phosphorylation of the motor protein was reported to change its localization and ATPase activity, or localization only, depending on the phosphorylated sites (2, 164).

Heat shock protein 70, a representative scaffolding protein, has also been implicated in interaction between KIF5 and cargo vesicles (226). The molecular mechanism is still unknown, but this reaction occurs in a MgATP-dependent and N-ethylmaleimide-sensitive manner, which may explain the two- to threefold increase in the yield of motor-associated vesicle population when brain samples...
are dissociated in buffer containing EDTA or N-ethylmaleimide.

After releasing their cargoes, motor proteins are supposed to be degraded at the destination, since little retrograde transport of anterograde motors has been detected within axons for reuse (69). An unknown mechanism including phosphorylation and degradation may function to clear up dissociated motor proteins.

C. Tug-of-War Between Motor Proteins

Anterograde axonal transport is conducted by various kinds of KIF, while cytoplasmic dynein is the only motor presently known to drive retrograde axonal transport. When real transport is observed in living axons, cargo vesicles do not necessarily move one-way. They are often carried back and forth within the axon and are sometimes stationary. This phenomenon is due to a tug-of-war between anterograde and retrograde motors, and the net result is that they are transported towards their own destination (145). Indeed, motor proteins of opposite directionality have been recognized as being attached to the same vesicles at the same time. Direct interactions between subunits of the dynactin complex and those of KIF complexes have also been reported; for example, an interaction between Glued p150 and KAP3 (26), or another between dynein intermediate chain and KLC (124). During movement in one direction, one motor must be turned on and the other off. How they are activated and inactivated alternatively remains unknown.

Some motors play multiple roles in transporting different kinds of cargoes. They sometimes have to choose different linking forms to bind appropriate cargoes, such as directly, via scaffolding/adaptor proteins and lipid. Among exogenously expressed cargoes, competitive interaction for a motor protein was observed. For example, Alcadein and JIP-1 are binding proteins for KLC and compete for KLC. More Alcadein was reported to be dissociated from KLC-KIF5 complex as the expression of JIP-1 increased (3). How a motor protein judges the order of priority of different cargoes and how it switches to another linking form for the appropriate cargoes still remain unclear. Moreover, in contrast to small cargoes like protein complexes, the size of vesicular cargoes is at most 10 times larger than that of motor proteins (Fig. 1). Vesicular membranes are supposed to contain other membrane proteins and lipids in addition to identified cargo proteins directly bound to the motor complexes. Whether motor proteins recognize such accompanied molecules and subsequently regulate their transport also needs to be examined in the future.

Fusion and fission of fluorescently labeled transported vesicles are commonly observed by time-lapse video microscopy. How motor proteins adapt themselves to a specific certain type of vesicle needs to be examined. It is also unknown how much a single motor protein contributes to driving its cargo along microtubules, whether it continues to bind cargo all the way to the destination or if it shifts to another motor and detaches from the cargo. Electron microscopy has revealed multiple crossbridges between cargo vesicles and microtubules (Fig. 1). Increasing data have also suggested cooperation among multiple motors in the transport of vesicles (11, 123). Together, biophysical analysis of the movement of cargoes and theoretical studies suggest that one to three anterograde motors on average simultaneously steer one vesicle in vivo. Further studies are needed to solve the regulatory mechanism controlling binding and unbinding of cargo, and motor activity.

X. STRUCTURE OF MOTOR PROTEINS

A. Structure of KIFs and Microtubules

In 1989, molecular structure of KIF5 was revealed to be composed of the globular motor domain, the stalk domain, and the tail domain (68, 190), and KLC was shown to bind the tail domain by immunoelectron micrographs decorated with an anti-KLC antibody (68). In 1995, three independent works clarified the three-dimensional structure at medium resolution (~30 Å) of microtubule-KIF complexes: microtubule-KIF5 and microtubule-Ncd, through computational analysis of cryo-electron micrographs (cryo-EM) (74, 76, 104). The authors of these studies not only showed that a motor domain of KIF5 is oriented to an identical region of a single protofilament, with an interval of 8 nm, but also clarified that Ncd, a C-kinesin, is oriented to the identical region to KIF5, irrespective of its original opposite directionality. In 1996, atomic structures of the motor domains of KIF5 and Ncd in the ADP-bound state were first solved by X-ray crystallography. Contrary to expectations, they revealed a structural similarity to myosin, although the amino acid sequences are apparently different between KIFs and myosin, and they show opposite kinetics to interact with microtubule or actin protofilament during the ATP hydrolysis cycles (117, 184). While KIFs are in the weak binding state when bound to ADP, myosin binds strongly to actin filament in the ADP-bound state. As deduced from the information about the cryo-EM and mutational studies, the core domains of microtubule interacting-interphase were identified as loop7/8, loop11, α4, and loop12/α5 (105, 165, 253).

After these works, several crystal structures of kinesin in the different subfamilies (kinesin-1, -3, -5, -13, and -14) have been solved, revealing that they have common structures of the catalytic core (106, 114, 117, 155, 162, 184, 204, 228, 253). Some differences in the flexible loop
regions presenting the difference of the length of the loops or different electric charges are expected to explain the diversity of the functions among kinesins of different subfamilies.

On the other hand, the atomic structure of tubulin was first reported by Nogales et al. in 1998 (160). Although this structure was solved using nonphysiological zinc-induced tubulin sheet, this work was the first report of the atomic structure of tubulin in the straight microtubule protofilament. In 2000, crystal structure of tubulin taking the curved conformation was solved in the form complexed with stathmin, which is one of the microtubule destabilizing MAPs (41). These two tubulin structures taking the straight and curved conformation are thought to represent the stabilized and destabilized form of microtubule, respectively. However, molecular mechanism of microtubule-dynamics is still unclear, and future studies are needed.

In the current stage, because of the lower resolution of the cryo-EM structure, in silico docking of the crystal structures of kinesin and tubulin to the medium-resolution cryo-EM structure of microtubule is the mainstream to construct the pseudo-atomic model of kinesin-microtubule complex. Recently, cryo-EM structures of KIF-microtubule complex have been obtained at much higher resolutions (9–12 Å) (73, 103, 203), and this technique is expected to have the potential ability to achieve the near-atomic resolution of kinesin-microtubule complex in the near future. Crystal structure of KIF-tubulin complex is also another potential candidate to reveal the real atomic structure of kinesin-microtubule complex.

B. Mechanism That Couples ATP Hydrolysis and Conformational Change

Similar to G proteins and myosins, kinesin holds nucleotide in the nucleotide binding pocket formed by well-conserved P-loop (amino acids 97–105 in KIF1A). An adjacent loop L9 (amino acids 199–218), which is called switch I from the analogy to G proteins, changes its conformation during the ATPase cycle, which is considered to trigger the hydrolysis reaction as well as the release of the product phosphate and the release and entry of nucleotide (Fig. 6). From the analogy to myosin, where nucleotide enters and leaves from one end of the nucleotide binding pocket and phosphate leaves from the other side, the conformational change related to the phosphate release is called as the opening of the “back door.” Recent structural studies have revealed details of this conformational change (155). Similarly, release of ADP and entry of ATP would take place through the “front door.” However, atomic structures of these states are currently missing, and little is known about the structural details of this process.

The hydrolysis reaction is tightly coupled to the conformational change of the switch I loop L9. How is this chemical cycle coupled to the mechanical cycle? The microtubule binding surface of kinesin is mainly composed of a series of structural elements loop L11-helix α4-loop L12-helix α5 (amino acids 248–324), which is called as switch II complex from the analogy to G proteins. Crystal structure of KIFs revealed that this complex takes two stable conformations: “up” and “down.”

In the “up” conformation, helix α4 protrudes on the surface so that it fits into the intradimer groove between α- and β-tubulins. Loop L11 extends toward tubulin, which contributes to the strong binding to microtubules. This is the “up” conformation; KIFs can bind to microtubules strongly (strong binding state).
In the “down” conformation, helix $\alpha 4$ is embedded between the groove by helix $\alpha 5$ and helix $\alpha 6$ so that the microtubule binding surface of kinesin become smooth and does not fit to the groove between the kinesin. Loop L1 is wound up to helix $\alpha 4$ so that it can no longer interact with tubulin. In this conformation, KIFs do not strongly bind microtubules (weak binding state).

Crystal structures by Nitta et al. (155) suggested that the conformational change of switch II complex is tightly coupled to that of switch I through formation and breakage of salt bridges between switch I and II. The “back door,” a key interaction for hydrolysis and phosphate release, is formed by the well-conserved salt bridge between switch I and switch II. Thus the formation and the breakage, namely, closure and opening, of the “back door” couples the hydrolysis reaction to the conformational change of the microtubule surface from “up” to “down” (strong binding to weak binding).

Upon the release of ADP and the following ATP binding, the reverse conformational change is expected to occur, from “down” to “up.” This conformational change would be coupled to the conformational change of switch I (“front door”). However, structural details are yet unclear due to the lack of the atomic structures of this state.

C. Processive Movement of KIF5

KIF5 is a highly processive motor, which hydrolyzes more than 100 molecules of ATP and translocates along microtubules successively before detaching from it (50, 80). Because monomeric constructs detach from microtubules after each cycle, dimerization is essential to move successively along microtubules for KIF5 (55, 56).

The “walking” model or a “hand-over-hand” model is now a widely accepted model of processive movement (Fig. 6) (249). In other words, a motor needs two legs, as we do in walking. When one foot steps forward, the other foot needs to be firmly attached to the rail for a motor to be able to “walk” without ever detaching from the rail. However, there has been a long controversy about the symmetricity of the walk, since the dimeric kinesin must walk along a protofilament with two identical right feet, as the structural data show. The “inchworm” model, that is, one head always leads the other head and moves like an inchworm, and the “asymmetrical hand-over-hand” mechanism, a dimeric motor translocates along a protofilament with alternately different step size, like limping, have been proposed (4, 81).

The first evidence for a hand-over-hand model was obtained from the experiment measuring the steps of single kinesin head labeled with Cy3 fluorescence, which presents the step size of 17.3 $\pm$ 3.3 nm, corresponding to the two steps of kinesin (250). Further evidence was then acquired from the conformational change of the necklinker synchronized with ATP hydrolysis (180). By adding electron paramagnetic resonance or a gold cluster tag to cysteine-333 in the necklinker, its movement during the ATP hydrolysis cycle was observed in both wild-type KIF5 as well as a low ATPase mutant construct. These data suggest that the necklinker extends towards the plus end while binding to ATP (Fig. 6), and then becomes mobile simultaneously with the release of $\gamma$-phosphate after ATP hydrolysis.

The neck region of KIF motors consists of two domains: a necklinker that attaches to the catalytic core, and a coiled coil region that unites two motors for dimerization (Fig. 6). Experiments inducing deletion or crosslinking into these regions clarified that a release of necklinker from the catalytic core is necessary for the processivity of motor proteins (182, 224). On the other hand, inhibition of the unwinding of the coiled coil region did not disrupt motility, although the overall length of two necklinkers is not sufficient for covering the step size of the motor. Instead of an unwinding effect, the positive charge of this region, which interacts electrostatically with the negatively charged COOH-terminal region of tubulin, rather contributes to the processivity of the motor (222, 224). Because monomeric KIF1A motor mainly uses this electrostatic mechanism to sustain its association to microtubules when in the weak binding state (165, 166), it is surprising that dimeric motor needs the same system, although either head is always connected to microtubules and protected from detachment. Recent work using single molecule FRET succeeded in examining the intermediate conformation of the heads between steps (144). At low-limiting concentration of ATP, the dimeric kinesin was in one-head bound state, where the detached head leaded another head attached to the microtubules (Fig. 6).

The mechanochemical coupling has also been investigated by a biophysics assay. To move one-directionally along a protofilament, two heads of dimeric KIF5 have to behave differently but coordinately depending on their relative positions. This “gating” mechanism was observed in both heads (13). ATP hydrolysis of the first head tightens the binding of the second front head to microtubules, which then accelerates the detachment of the first head for forward displacement steered by the power-stroke of second head (35, 55). The gating mechanism is supposed to be mediated by the reciprocal mechanical strain through the direct or indirect connection of the heads via the necklinker and protofilament (13). How the strain then affects the binding and hydrolysis will be clarified in the future.

D. Structure of C-Kinesins

In addition to the fundamental mechanism common to KIF5, other KIFs play specific roles in vivo by using...
their specifically modified structures. In the following chapters, three examples, Ncd, KIF1A, and KIF2C, are discussed.

C-kinesins, which have a motor domain in the COOH terminus of the molecules, drive minus-end-directed motility. Because of their opposite directionality to other N-kinesins, they have been studied for the mechanism determining the directionality of motors. The three-dimensional structure of the Ncd-microtubule complex was first reported in 1995, along with KIF5 (74, 75, 184, 207). Despite opposite directionality, the catalytic core of Ncd binds to the identical site in the microtubule to KIF5. To determine which domain is essential for directionality, replacement of a domain of KIF5 with a corresponding Ncd domain, or induction of a point mutation within the region concerned, has been performed, showing that directional bias is determined by the neck/core interaction but not by the catalytic core itself (20, 34, 61, 183). Indeed, a mutant Ncd with a single amino acid change in the neck region presented reverse motility, which became then bidirectional by inducing another mutation in the facing catalytic core (34). When a three-dimensional structure of dimeric Ncd with microtubules was solved by cryo-EM and computational analysis, the unbound second head tilted towards the minus end, while the unbound KIF5 head oriented to the plus end (75), reflecting their original directionality.

Analysis of the crystal structure of Ncd revealed that the neck region of Ncd does not have a necklinker like KIF5, but that it consists of a solid α-helix, although the catalytic core of Ncd is located in the similar orientation to microtubules to that of KIF5 (183). Moreover, displacement of Ncd along microtubules seems biased clockwise, while directed towards the minus end (34). Molecular biophysics experiments have shown that Ncd has a mechanochemical cycle that is totally different from that of KIF5 (27). Ncd does not move in consecutive 8-nm steps like KIF5, but, on average, about a 9-nm large axial displacement synchronized with ATP capture and tends to detach from the microtubules, showing the characteristics of a nonprocessive motor (27, 37). Thus some drastic difference between Ncd and KIF5 may be derived from the different arrangement of the neck. To obtain the details by biophysiological data on its motility, discovery of a processive type of C-kinesin motor is awaited.

E. Monomeric Motor KIF1A: How Does It Move?

In contrast to other motor proteins, such as KIF5, Ncd, and myosin, KIF1A exists as a monomer in solution (168). Therefore, it cannot move processively by the "hand-over-hand" mechanism (56). However, the shortest motor domain construct of the KIF1A molecule, C351, moved along a microtubule processively over a distance of more than 1 μm (166). On average, C351 hydrolyzed nearly 700 ATP molecules before detaching from the microtubule, which is more than 7 times larger than KIF5.

In contrast to KIF5, however, the movement of single C351 molecules along the microtubule was not smooth and actually appeared to be oscillatory. Quantitative analysis of this apparently oscillatory movement revealed a linear increase in mean displacement and mean variance, indicating a unidirectional constant-velocity movement accompanied by Brownian movement (5). When comparing the movement of C351 with that of K381, a dimeric construct of a conventional KIF5 molecule, plots of mean square displacement against time [[MSD, σ(t)]] from C351 fit well with biased Brownian movement (166).

The presence of the diffusional component in the movement of KIF1A suggests that KIF1A is loosely attached to microtubules even in the state in which other KIFs are detached from microtubules. Thus a single-headed KIF1A would be able to advance without fully detaching from microtubules. Indeed, a monomeric KIF1A construct C351 had higher affinity to microtubule even in the ADP state, when other KIFs are in the weak-binding state. C351 in ADP state, however, was not stably bound to microtubules but showed a one-dimensional Brownian movement along the microtubules (166).

In search for the loose anchor for KIF1A, a class-specific extension in the loop L12 was noticed. This extension contains five successive lysine residues so that it is named "K-loop." This highly positively charged loop extends toward microtubules in the ADP state. The K-loop is supposed to interact with the negatively charged domain of tubulin COOH-terminal named the "E hook," which contains many glutamates (165, 166). Mutation constructs with fewer lysines in the K-loop produced a significantly decreased processivity of the motor (165), suggesting that the positive charge of the K-loop contributes to the affinity of KIF1A for microtubules.

F. Structure of KIF2C/MCAK: A Common Mechanism for Microtubule Destabilization

KIF2 depolymerizes microtubules at the ends of microtubules. Within cells, however, KIF2 does not necessarily exist only at microtubule tips, but usually binds to the sides of microtubules and moves rapidly towards the ends by diffusion through an interaction between the positively charged neck and negatively charged E-hook of tubulin (83). However, in contrast to KIF1A, this one-dimensional diffusion has no directional predominance and does not require the hydrolysis of ATP (28, 60, 83). The deletion of the positively charged residues in the neck domain of KIF2C reduces the microtubule depolymerizing activity of KIF2C, which is then rescued by the substitu-
TABLE 1. *Cargo complex transported by KIFs*

<table>
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KIF, kinesin superfamily protein; KLC, kinesin light chain; KAP3, kinesin superfamily-associated protein 3; IFT, intraflagellar transport.
tion of this neck with K-loop of KIF1A (171). Thus the electric interaction with the negatively charged E-hook of tubulin seems generally used by KIFs to sustain its affinity for microtubules. Upon arriving at the ends, KIF2C/MCAK hydrolyzes multiple ATPs and removes, on average, ~20 tubulin dimers before finally dissociating from the end. This characteristic behavior of KIF2 seems to be derived from its molecular structure as an M-kinesin. The interaction of KIF2 with microtubules is expected to provide another structural hint regarding the function of KIFs.

The crystal structures of KIF2C in the ADP- and AMP-PNP-bound states were investigated to reveal the molecular mechanism underlying microtubule depolymerization (162, 204). The crystallized monomeric construct includes a neck region that is located NH2-terminal to the motor domain in the M-kinesin. In contrast to other KIFs, KIF2C shows a particular conformation as an M-kinesin (162). A neck region of KIF2C forms an α-helix-rich structure extending vertically downward to the microtubules. The loop2 domain of KIF2C protrudes like a
unique finger structure and is named the “KVD finger.” Second, even in the ATP-like (AMP-PNP-bound) state, the nucleotide binding pocket in KIF2C is open independently of the bound nucleotide.

When the atomic model of KIF2C was fitted to the microtubule protofilament, the microtubule binding surface of KIF2C was too convex to fit to the straight protofilament on the shaft, but it does fit well on the curved protofilament at the ends (162). Thus KIF2C does not bind tightly to the side wall of microtubules but diffuses and binds tightly at the end (83). At the curved ends, the KVD finger inserts into the inter-dimer interface of tubulin and stabilizes the curved conformation of the protofilament (162). In order for KIF2C to bind more tightly to the curved protofilament, L8 is pushed up and causes the rotation of the α3 helix and, subsequently, the α2 helix; this rotation synchronously induces the closing of the nucleotide-binding pocket and starts ATP hydrolysis. NH2-terminal neck plays an additional role in extending into the side groove of the protofilament and destabilizes the lateral interaction of adjacent protofilaments, which in turn enhances the dissociation of tubulins. The final closure of the nucleotide binding pocket induced by the passive conformational change of the motor through an interaction with protofilament is novel to KIF2C.

XI. CONCLUSIONS AND FUTURE PERSPECTIVES

Cells use various types of KIF motor, including monomeric-type, dimeric-type, heterodimeric-type, NH2-terminal motor domain-type, middle motor domain-type, and COOH-terminal motor domain-type motors (Figs. 2 and 3). Such motors transport various molecules in the form of membrane vesicles, protein complexes, and mRNA-protein complexes to their proper destination, at a proper velocity (Table 1). In this review, each type of molecular transport implicated by KIF motors has been described first in terms of the route taken, the particular cargoes being transported, and the particular motor proteins involved ( sects. iii-vii) (Fig. 4). In many routes, multiple motor proteins play complementary, redundant parts to accomplish the transport process within cells. At a higher level, such as in tissues and individual animals, motor proteins have been implicated in determining cell morphology, cell-cell contacts, and differentiation (Fig. 7). KIFs even control important events such as left-right axis determination during development, brain wiring, activity-dependent neuronal survival, suppression of tumor formation, and higher cortical functions, such as learning (sect. viii). Second, interactions between molecules are mentioned. To execute multistage transport processes, the intermolecular interaction between motor and cargo is indispensable. Accumulating evidence has just begun to reveal an elaborate mechanism for the recognition and regulation of intracellular transport (sect. ix). Third, the intramolecular mechanism underlying the conformational change in motor proteins is also discussed, through analysis of the crystal structures and the cryo-EM images of motor proteins (sect. x).

The intracellular “physical distribution system” sets up widespread networks within cells and manages proper execution of totally balanced transport. We still do not understand very well the mechanism involved. There are still many KIFs whose functions are not clarified. We do not yet fully know how motor molecules recognize and bind to their cargoes, how they choose a particular transport route and, finally, how they identify the destinations for their cargoes. Besides, in addition to KIFs, myosin superfamily proteins and dynein superfamily proteins are also involved in intracellular transport (65, 229). It is still unknown how they recognize one another and how, on certain occasions, they take over the cargoes or cooperate to accomplish this task. As for a supervision mechanism to coordinate transport by multiple motors, we have no knowledge at all. Further experiments will reveal the detailed mechanism of intracellular transport in the future.

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