integrity. The recent discovery of linker proteins bridging between IFs and other cytoskeletal components and their importance to cell survival and genetic disease open a new field for understanding the functional interactions among the structural elements within the cytoplsm.

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Kinesin and Dynein Superfamily Proteins and the Mechanism of Organelle Transport

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Cells transport and sort proteins and lipids, after their synthesis, to various destinations at appropriate velocities in membranous organelles and protein complexes. Intracellular transport is thus fundamental to cellular morphogenesis and functioning. Microtubules serve as a rail on which motor proteins, such as kinesin and dynein superfamily proteins, convey their cargoes. This review focuses on the molecular mechanism of organelle transport in cells and describes kinesin and dynein superfamily proteins.

Neurons and epithelial cells are among the many types of cells that develop polarized structures. The neuron is composed of a cell body, dendrites, and a long axon along the direction of impulse propagation. The axon lacks protein synthesis machinery, and thus all the proteins required in the axon and synaptic terminal must be transported down the axon after they are synthesized in the cell body. Most proteins are conveyed in membranous organelles or protein complexes. In this sense, organelle transport in the axon is fundamentally important for neuronal morphogenesis and functioning. Because similar mechanisms are observed in other cells, the neuron serves as a good model system to study the general mechanisms of organelle transport (*1*). Epithelial cells also develop polarized structures, that is, the apical and basolateral regions, to which certain proteins are specifically transported and sorted (*2*).

Microtubules are 25-nm tubule-like structures formed by α,β-tubulin heterodimers. Thirteen parallel protofilaments composed of linearly arranged heterodimers form the

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microtubule wall, to which various microtubule-associated proteins and motor proteins bind. The microtubule is a polar structure with a fast-growing or plus end and a minus end.

Microtubules serve as rails for the transport of organelles and are organized in a regular manner in these polarized cells. In nerve axons, the microtubules are arranged longitudinally with the plus end pointing away from the cell body, whereas in epithelial cells microtubules are organized with the plus end pointing toward the basement membrane. In most other cells such as fibroblasts and macrophages, microtubules radiate from the cell center with the plus ends pointing toward the periphery. In all of these cells, various organelles are transported along the microtubules by means of microtubule-associated motor proteins.

Early light microscopy studies of living nerve axons and biochemical studies of axonal transport revealed membranous organelles moving by fast flow (3). Electron microscopy (EM) studies suggested the presence of short cross-bridge structures between the organelles and microtubules, which are candidates for microtubule-associated motor proteins conveying the membranous organelles along microtubules (4) (Fig. 1). Video-enhanced differential interference contrast microscopy combined with biochemical analyses revealed the presence of a microtubule plus end–directed motor protein, kinesin, a microtubule-activated adenosine triphosphatase (ATPase) of 380 kD (5, 6). The kinesin molecule consists of two 120-kD kinesin heavy chains (KHCs) and two 64-kD kinesin light chains (KLCs) (6). It has a rod-like structure composed of two globular heads (10 nm in diameter), a stalk, and a fan-like end, with a total length of 80 nm. The globular heads are composed of KHCs that bind to microtubules (7, 8) (Fig. 2); the KLCs constitute the fan-like end (7). Complementary DNA (cDNA) encoding *Drosophila* KHC yields a protein of 975 amino acids in which the NH_2-terminal ~350 amino acids form the motor domain (which binds to microtubules), an α-helical coiled coil–rich stalk domain involved in dimer formation, and a tail domain (9). Localization and functional assays indicate that kinesin acts as a plus end–directed microtubule

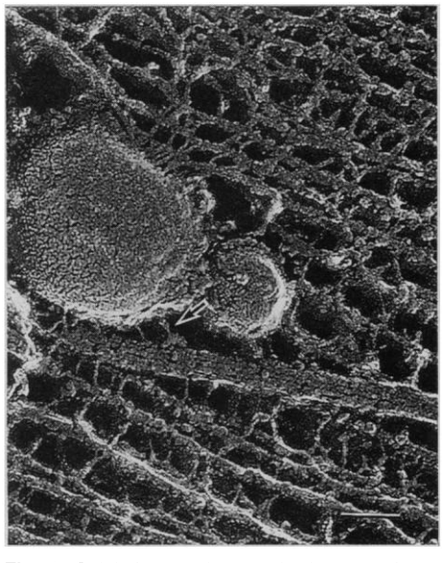


Fig. 1. Quick freeze–deep etch electron micrograph of mouse axon. A membranous organelle conveyed by fast transport is linked with a microtubule by a short cross-bridge (arrow), which could be a motor molecule. Scale bar, 50 nm.

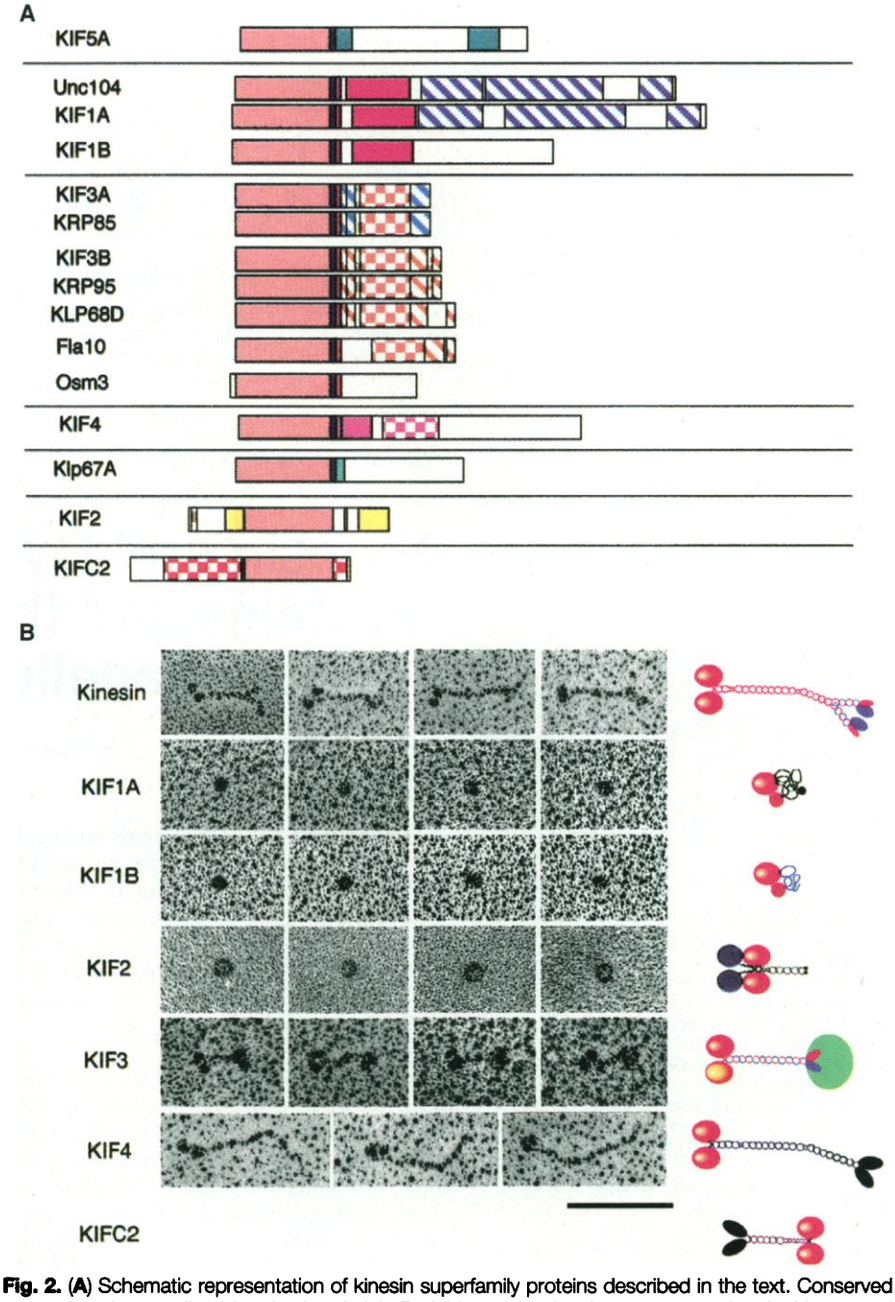


Fig. 2. (**A**) Schematic representation of kinesin superfamily proteins described in the text. Conserved motor domains are aligned and colored pink. Dark blue and red regions correspond to the N-type and C-type (KIFC consensus) specific neck regions, respectively. Yellow rectangles indicate the M-type specific neck and tail regions. Other conserved regions within each class, family, or subfamily are indicated by solid rectangles, oblique stripes, or checks, respectively, with different colors. (**B**) Left: Panels of main members of KIFs functioning in organelle transport, as observed by low-angle rotary shadowing EM. Scale bar, 100 nm. Right: Schematic illustrations of the same KIFs, based on EM studies or predicted from analysis of primary structures.

motor involved in anterograde membrane transport (*10–12*).

Another microtubule-activated ATPase that promotes transport in the opposite direction, known as brain dynein or cytoplasmic dynein, was discovered in 1987. Cytoplasmic dynein is composed of two heavy chains of 530 kD each, three intermediate chains of 74 kD each, and four light intermediate chains of 55 to 60 kD each, and moves along a microtubule from the plus end to the minus end, making it a good candidate for a motor for retrograde axonal transport (*13*). In living axons, however, various types of membranous organelles (including synaptic vesicle precursors and vesicles containing synaptic and axonal plasma membrane precursors) are transported anterogradely, whereas multivesicular bodies and endosomes are transported retrogradely, with both types of transport occurring at distinct velocities. Mitochondria are transported in both directions (*1, 3*).

The kinesin superfamily of proteins plays a major role in this complex organelle transport. A systematic molecular biological search of kinesin superfamily genes coding for proteins containing adenosine triphosphate (ATP)–binding and microtubule-binding consensus sequences led to the discovery of new kinesin superfamily proteins related to organelle transport (KIFs), 11 from mouse brain (*1, 14*) and three from *Drosophila* (*15*). Motor proteins from *Caenorhabditis elegans* were identified in mutants with slow and uncoordinated movement [for example, Unc104 (*16*)] or chemotaxis [Osm3 (*17*)]. Further motor proteins ($KRP_{85/95}$) have been identified in biochemical extracts from sea urchin (*18*). Systematic molecular biological searches have identified at least two or three members of the dynein superfamily proteins related to the transport of organelles in sea urchin (*19*), rat (*20*), and human (*21*).

In this review, I describe well-characterized kinesin and dynein superfamily proteins and their function in organelle transport in cells. Some other members of these superfamilies are also involved in mitosis or meiosis. [See (*22, 23*) for comprehensive recent reviews of the motor proteins involved in cell division and the mechanism of motility of motor proteins.]

The Kinesin Superfamily Proteins

Three major types of kinesin superfamily proteins have been identified according to the position of the motor domain: NH_2-terminal motor domain type, middle motor domain type, and COOH-terminal motor domain type (referred to below as N-type, M-type, and C-type, respectively). Of the proteins that have been identified, the KHC, Unc104/KIF1, KIF3/$KRP_{85/95}$, KIF4, and Klp67A families (N-type), the KIF2 family (M-type), and the KIFC2/C3 family (C-type) are involved in organelle transport (Figs. 2 and 3).

N-type proteins: Conventional kinesins. Conventional KHC itself forms a family (Fig. 3). Although three members of this family have been identified in mouse (KIF5A, KIF5B, and KIF5C) (*1, 14, 24*) and two in humans (HsuKHC and HsnKHC) (*25*), only one member has been identified in other metazoans such as sea urchin, *Drosophila*, and *C. elegans* (*26*). KIF5B and HsuKHC are expressed ubiquitously in many tissues, whereas KIF5A, KIF5C, and HsnKHC are specific to nerve tissue.

Kinesin is primarily associated with anterogradely transported membranous organelles in nerve axons (*11*). In various types of cells, kinesin associates with the endoplasmic reticulum (ER), Golgi complex, mitochondria, endosomes, and lysosomes (*22*). Several distinct approaches have been adopted to elucidate the function of kinesin. The *Drosophila* KHC gene mutants do not survive beyond the larval stage and exhibit loss of mobility and tactile response. Thus, KHC in *Drosophila* transports proteins important for action potential propagation, whereas KHC mutants exhibit no apparent change in the concentration of synaptic vesicles in nerve terminal cytoplasm (*26*).

Various kinds of functional studies, including antisense studies (*27*), microinjection of blocking antibodies (*12, 28, 29*), and transfection of KHC cDNA containing a point mutation of the ATP-binding domain (*30*), have resulted in a variety of phenotypes in cells, sometimes conflicting with each other (*27–29*). In summary, however, it is reasonable to conclude that kinesin is responsible for the microtubule plus end–directed transport of membranous organelles, which is important for the neurite outgrowth of neurons, and for microtubule plus end–directed lysosome transport in various types of cells. Further studies are needed to characterize the cargoes of kinesin in vivo.

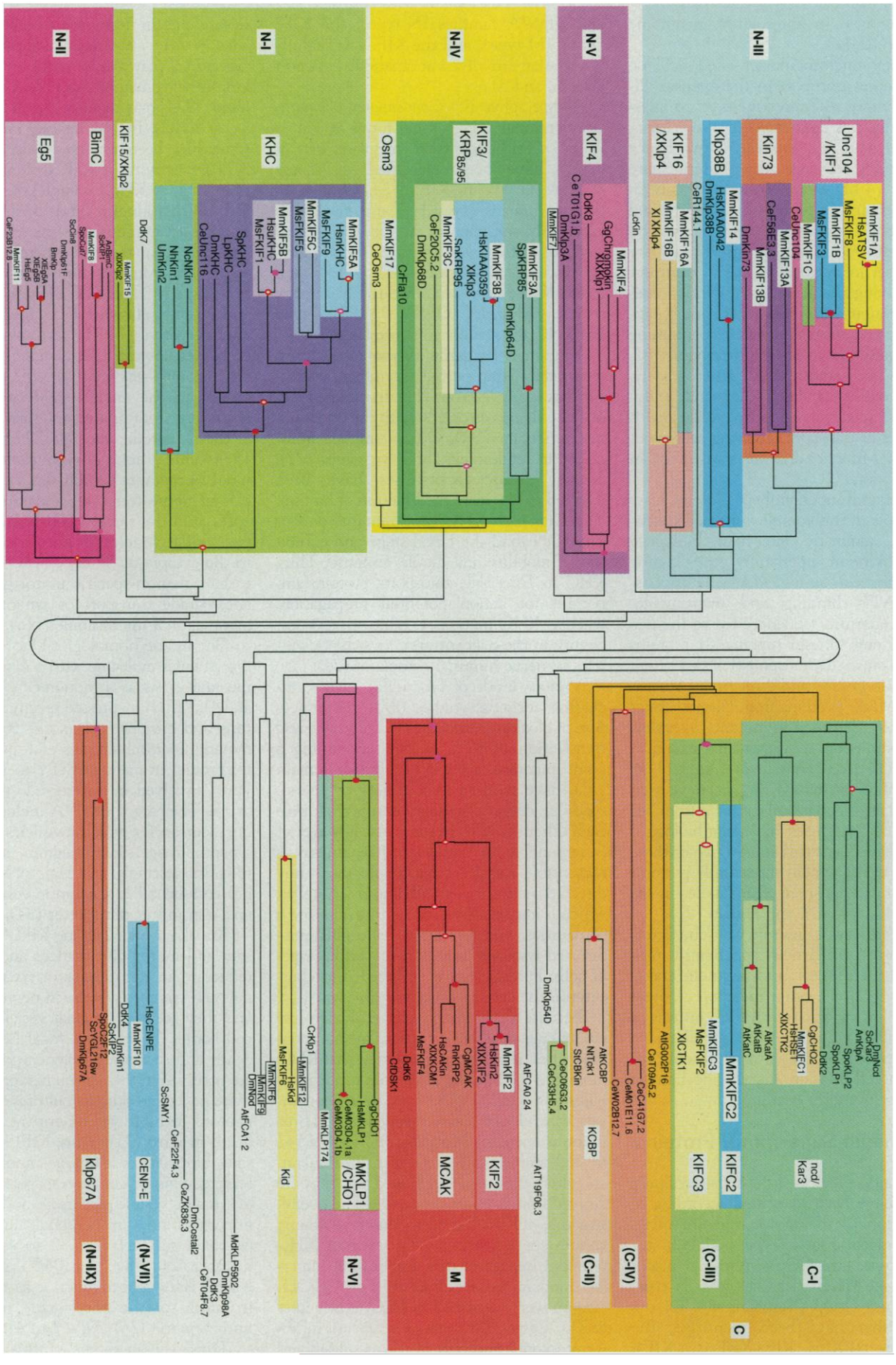
Because KLCs are localized at the fan-like end of kinesin where it binds to membranous organelles, it has been speculated that KLCs modulate the binding of cargoes to microtubules (*7*). KLC cDNAs from several organisms were cloned and sequenced (*31*). Three isoforms encoding peptides composed of 542 to 560 amino acids were identified in rat, and alternative splicing showed that they are derived from a single gene (*31*). The overall structure of KLC has been conserved among various species, and a long series of NH_2-terminal heptad repeats and several imperfect tandem repeats closer to their COOH-termini were identified in KLC. Antibodies to KLCs, especially to tandem repeats, inhibit fast axonal transport in the axoplasm and release kinesin from purified membrane vesicles in vitro; this finding supports the idea that KLCs play a role in the interaction of kinesin with membranes (*32*). On the other hand, KLCs may regulate the ATPase activity of KHCs. In EM images, kinesins have sometimes been observed folded (*7*). The ATPase activity of KHCs alone is greater than that of KHCs complexed with KLCs (*33*). When kinesin binds membranous organelles to microtubules, its head domains are apart from the tail, whereas in the absence of microtubules kinesin may be folded, bringing KHC and KLC close to each other and possibly allowing KLC to regulate the ATPase activity of KHC in vivo (*33*).

N-type proteins: Fast anterograde monomeric motors—the Unc104/KIF1 family. Mutations in the *unc104* gene of *C. elegans* result in uncoordinated and slow movement of the nematode; *unc104* cDNA encodes a 1584–amino acid N-type motor protein. Unc104 mutants exhibit few synaptic vesicles in nerve terminals and form few synapses, and the neuron cell bodies accumulate similar vesicles tethered together within the cytoplasm. Thus, Unc104 is thought to be a neuron-specific motor used for the anterograde transport of synaptic vesicles along axonal microtubules (*16*).

The mouse homolog of Unc104, KIF1A, is a globular molecule with a diameter of 14 nm and exists as a monomer (*34*) (Figs. 3 and 4). KIF1A, expressed specifically in neurons and enriched in axons, is the fastest moving microtubule plus end–directed motor protein in mammals (1.2 to 1.5 μm/s in vitro) involved in anterograde transport in axons. The cargo of KIF1A includes a subset of precursors for synaptic vesicles containing synaptophysin, synaptotagmin, and Rab3A (*34*). Interestingly, the cargo of KIF1A does not contain SV2, a synaptic vesicle protein and transmitter transporter (*34*).

Knockout mice lacking KIF1A show sensory and motor disturbances and a marked reduction in the density of synaptic terminals and synaptic vesicles in nerve terminals, and they accumulate clear vesicles in nerve cell bodies. In addition, focal neuronal cell death and secondary degeneration of axons are observed in the central nervous system of these mice, and primary cultures of $KIF1A^{-/-}$ neurons exhibit prominent neuronal cell death in vitro (*35*). Thus, KIF1A (and possibly Unc104) is a unique monomeric anterograde motor for the transport of a subset of synaptic vesicle precursors, and it plays an important role in neuronal function and neuronal survival (Figs. 2 to 4). Another member of the Unc104/KIF1A family, KIF1B, is thought to function as a monomeric motor for the anterograde transport of mitochondria (*36*) (Figs. 2 to 4).

Thus, the members of this family are


N-III
Unc104 /KIF1
Kin73
Klp38B
KIF16 /XKlp4
N-V
KIF4
N-IV
KIF3/ KRP85/95
Osm3
N-I
KHC
N-II
Eg5
BimC
KIF15/XKlp2
C-I
ncd/ Kar3
KIFC2
C-III
KIFC3
C-IV
C-II
KCBP
C
M
MCAK
KIF2
MKLP1 /CHO1
N-VI
Kid
CENP-E
N-VII
Klp67A
N-IIX

mostly monomeric microtubule plus end–directed motor proteins with a variety of distinct cargoes. How this monomeric single-headed motor moves on a microtubule is an important and intriguing question, because the hand-over-hand mechanism cannot be used, as with the dimeric, two-headed motors (*1*, *37*).

N-type proteins: Anterograde heterodimeric motors—the KIF3/KRP$_{85/95}$ family. Another group of two-headed anterograde motors includes the KIF3/KRP$_{85/95}$ family (Figs. 2 and 3). A KIF3A-KIF3B heterodimer (KIF3A/3B) assembles with KAP3 (kinesin superfamily associated protein–3), forming a heterotrimeric motor with a plus end–directed microtubule sliding activity at a velocity of ~0.3 μm/s (*14*, *38*, *39*). The motor is expressed ubiquitously and is used for the anterograde transport of membranous organelles, 90 to 160 nm in diameter, distinct from synaptic vesicle precursors and from vesicles carried by other motors such as kinesin and KIF2 (*38*) (Figs. 2 to 4).

KAP3 is a globular protein (~11 nm in diameter) that binds to the tail domain of KIF3A/3B. KAP3 binding does not affect the ATPase activity of KIF3A/3B, but it is associated with the membrane-bound form of KIF3A/3B (through the tail domain of KIF3A/3B) and is thought to regulate the membrane binding of the KIF3 heterodimer (*39*) (Fig. 2). The human homolog of KAP3 has been shown to be a small–molecular weight G protein GDP dissociation stimulation factor (SmgGDS)–associated protein that is phosphorylated by Src tyrosine kinase and regulates the interaction of a group of small G proteins with membranes (*40*). The *Drosophila* homolog of KIF3B, Klp68D, is also thought to play a role in anterograde axonal transport (*41*). The sea urchin homolog of KIF3A/3B, KRP$_{85/95}$, in association with KAP115 (also named kinesin II) (*42*), is thought to deliver ciliary components, most likely protein complexes that are required for the elongation of cilia and for the formation of a stable central pair of cilial microtubules (*43*) (Fig. 3).

In *Chlamydomonas*, analysis of *fla10* mutants defective in flagellar assembly led to the identification of the *fla10* gene encoding another member of the KIF3/KRP$_{85/95}$ family, KHP1 (Fla10) (*44*) (Figs. 2 and 3). KHP1 is required for the movement of particles within flagella and for the transport of inner dynein arms, subunit p28^{IDA4}, to the tip of flagella (*44*, *45*).

A final member closely related to the KIF3/KRP$_{85/95}$ family is exemplified by the *C. elegans osm-3* mutant, which has defects in chemosensory responses such as osmotic stress avoidance and chemotaxis (*17*). The dendritic sensory cilia of certain groups of sensory neurons in amphid and phasmid sensilla are foreshortened. Osm3 probably transports materials necessary for the growth of sensory cilia in sensory neurons (*17*) (Fig. 3). In summary, motors of this family (KIF3A/3B, KRP$_{85/95}$, and probably Klp68D/64D) form a heterotrimer with an associated protein KAP.

N-type proteins: KIF4 and Klp67A families. Another family of motors is illustrated by KIF4 (*14*, *46*). KIF4 messenger RNA (mRNA) is expressed abundantly in juvenile tissues, including differentiated young neurons; in adult mice, its expression is considerably decreased, except in the spleen. KIF4 is colocalized with membranous organelles in the growth cones of differentiated neurons as well as in the cytoplasm of cultured fibroblasts. During the mitotic phase of the cell cycle, KIF4 appears to colocalize with membranous organelles in the mitotic spindle. Hence, KIF4 is a microtubule plus end–directed anterograde motor for the transport of a certain group of membranous organelles in juvenile neurons and other cells (*46*) (Figs. 2 to 4). Chromokinesin, the chicken isolog of KIF4, contains a basic leucine zipper DNA binding domain, is associated with chromosome arms, and functions as a mitotic motor with DNA as its cargo (*47*) (Fig. 3). Because KIF4 also contains a DNA binding domain similar to that of chromokinesin, KIF4 may have an additional function such as the transport of mRNA in certain kinds and developmental stages of cells.

A final N-type motor is *Drosophila* Klp67A. It is a microtubule plus end–directed motor (0.05 μm/s) (Figs. 2 and 3) that acts as a mitotic motor and may play a role in positioning mitochondria near the mitotic spindle pole (*48*).

M-type proteins: The KIF2 family. KIF2 (716 amino acids, relative molecular mass M_r = 80,945) is a unique M-type KIF (*14*, *49*). The NH_2-terminal domain of 189 amino acids is predicted to be globular and the central motor domain is also predicted to be largely globular, whereas the COOH-terminal region is predicted to be largely α-helical. KIF2 forms a homodimer, a globular molecule with a diameter of ~16 nm (Figs. 2 and 3). KIF2 is a microtubule plus end–directed motor (~0.4 μm/s) and is expressed ubiquitously. It is abundantly expressed in developing axons, whereas the extent of expression decreases in adults. The cargo of KIF2 is vesicles about 100 to 120 nm in diameter, enriched in the neuronal growth cone and distinct from the membranous organelles carried by other motors (*49*). The cargo of KIF2 includes the specific form of β subunit of the insulin-like growth factor–1 (IGF-1) receptor (βgc), and the treatment of PC12 cells with KIF2 antisense RNA inhibits neurite outgrowth in PC12 cells (*50*). Thus, KIF2 is a unique M-type KIF anterogradely transporting vesicles important for axonal extension in developing neurons (Figs. 2 to 4).

C-type proteins: The KIFC2/C3 family. Several C-type motors, such as ncd in *Drosophila* and Kar3 in *S. cervisiae*, are motors for meiosis, mitosis, and karyogamy (*51*) (Fig. 3). These family members show microtubule minus end–directed motility. Because the only microtubule minus end–directed motor identified for organelle transports is cytoplas-

Fig. 3. (facing page) Phylogenetic tree of kinesin superfamily proteins. Superclass, class, family, and subfamily designations are based on molecular phylogenetic analysis of information derived from the motor domain and multiple sequence comparisons of the nonmotor domain of each kinesin superfamily protein, as follows: The superclass was defined by the existence of the conserved neck consensus (~10 amino acids) adjacent to the motor domain. For many KIFs, this superclass corresponds to the position of the motor domain in the molecule; superclasses N, M, and C correspond to N-type, M-type, and C-type KIFs. Class was defined by the class-specific consensus domains or regions that are often found adjacent to the superclass consensus neck. Eight classes (N-I, N-II, N-III, N-IV, N-V, N-VI, N-VII, and N-IIX) in superclass N, one class (M) in superclass M, and four classes (C-I, C-II, C-III, and C-IV) in superclass C were identified. Class numbers and family and subfamily names are derived from the time of discovery and characterization of the genes. Sequences of KIFs registered in the public databases (GenBank/EMBL/DDBJ DNA database or PIR/SwissProt/PRF protein database) by 27 August 1997 were analyzed by Clustal alignment and the neighbor-joining method. (Details of the phylogenetic analyses are available on our World Wide Web page, http://cb.m.u-tokyo.ac.jp/KIF.) The name of each KIF consists of the acronym of the species name followed by its conventional name or registered name in the database. For the sequences identified in the genome project, the cosmid name is used. For example, mouse (*Mus musculus*) protein KIF1A is designated as MmKIF1A, and the *C. elegans* sequence found as the third protein coded in cosmid F56E3 is designated as CeF56E3.3. Thus, it is easy to access database entries using these names and the program DBGET; accession numbers are also available on our Web page. Nodes with >950 of 1000 bootstrap values are marked with solid red circles; nodes with >900 of 1000 bootstrap values are marked with open red circles. The clusters identified with this analysis are color-coded. For example, the large light blue rectangle at the upper left marks class N-III, which contains Unc104/KIF1 (pink), Kin73 (orange), Klp38B (blue), and KIF16/XKlp4 (salmon) families; in turn, the Unc104/KIF1 family consists of three subfamilies: KIF1A (yellow), KIF1B (blue), and KIF1C (green). Names of families and their members referred to in this review and related to organelle transport are in larger type. For the family name, the name of the founding member (the first cloned and characterized member) is adopted.

mic dynein, it is reasonable to assume that as yet unidentified C-type KIFs exist for retrograde transport. On the basis of the discovery of a COOH-terminal KIF consensus sequence at the neck region upstream of the head region (*52*) and the results of a polymerase chain reaction using a motor domain gene consensus sequence (*52*, *53*), three C-type KIFs have been identified in mouse brain (*24*, *52*–*54*). KIFC2 forms a homodimer without associated polypeptides. It exhibits a microtubule-activated ATPase activity (*51*, *52*) and is localized mainly in the cell body and dendrites (*52*). The cargo of KIFC2 was identified as a new kind of multivesicular body–like membranous organelle, distinct from conventional multivesicular bodies, that functions as a shuttle between early and late endosomes (*52*). Because KIFC2 is mainly localized in the cell body and dendrites, even after the overexpression of KIFC2 in primary cultured neurons, it is thought to be a unique C-type motor that mainly functions in the dendritic transport of multivesicular body–like membranous organelles (Figs. 2 and 4). The amino acid sequence analysis of KIFC2 strongly suggests that it moves along microtubules toward the minus end, but this cannot be firmly established without an in vitro motility assay (*52*, *53*).

New KIFs. Further KIFs are likely to play roles in organelle transport, such as KIFs conveying synaptic plasma membrane proteins essential for vesicle docking (for example, SNAP25 and syntaxin 1A), KIFCs as retrograde motors in axons, and slow transport motors carrying cytoskeletal proteins and cytosolic proteins. A systematic search for cDNAs encoding as yet unidentified KIFs has been performed, revealing a total of 18 new KIFs (KIF3C, 6, 7, 8, 9, 10, 11, 12, 13A, 13B, 14, 15, 16A, 16B, 17, 22, C3, and C4) (*24*, *54*) (Fig. 3). On the basis of the expression of their mRNA in tissue, these KIFs could play important roles in organelle transport in many types of cells, including neurons and epithelial cells.

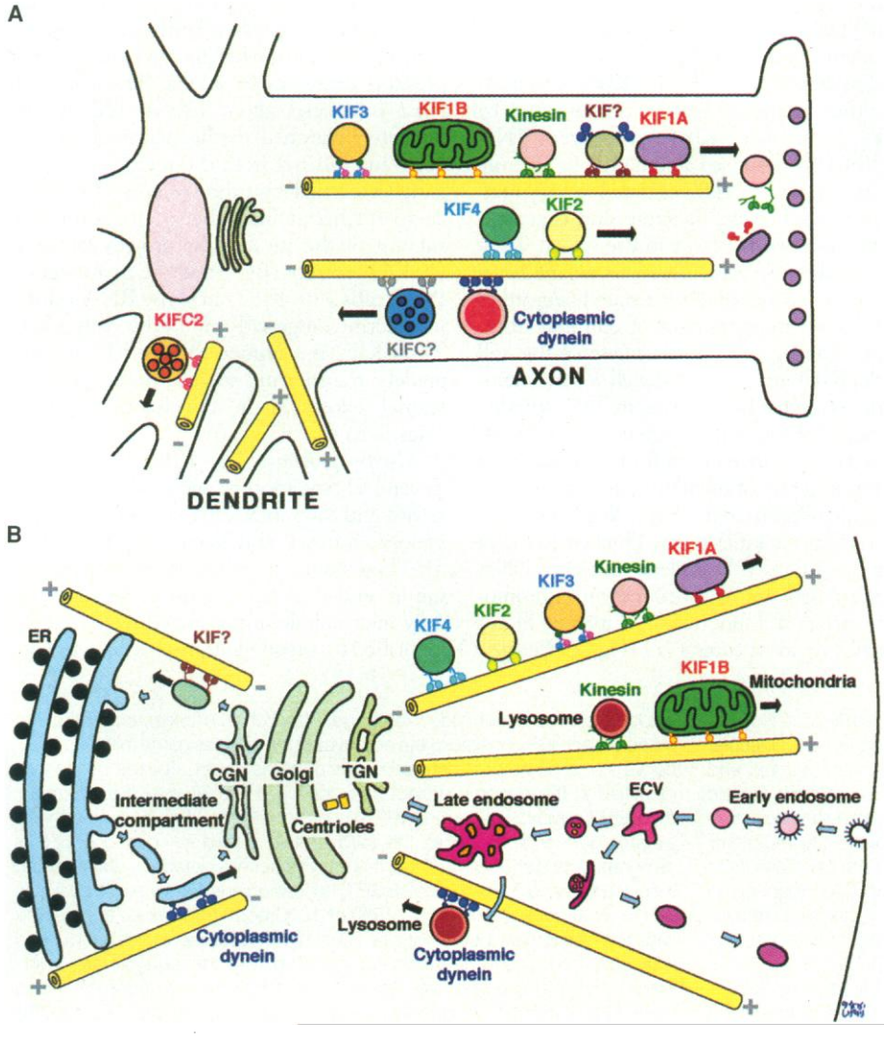


Fig. 4. Scheme of KIFs and cytoplasmic dynein and their cargo organelles in nerve axons (**A**) and in cells in general (**B**). In neurons, KIF2 and KIF4 work mostly in juvenile stages. In (B), neuron-specific KIFs and ubiquitous KIFs are drawn in the same cell. CGN, cis-Golgi network; TGN, trans-Golgi network; ECV, endosomal carrier vesicle. Black arrows indicate the direction of transport.

The Dynein Superfamily Proteins

Cytoplasmic dynein is a member of the dynein superfamily of proteins. It is a massive multisubunit complex (1.2 MD) composed of two heavy chains (~530 kD), three intermediate chains (74 kD), and four light intermediate chains (~55 kD), and moves along microtubules toward their minus ends (*13*, *23*) (Fig. 5). The cytoplasmic dynein heavy chain consists of 4644 amino acids in rat and 4092 amino acids in *Dictyostelium* and yeast, and it contains phosphate-binding pockets (P-loops) in its central region (*23*, *55*). On the basis of a comparison of the primary structure of cytoplasmic dynein with that of axonemal dynein, the central and COOH-terminal regions are predicted to form a globular domain interacting with microtubules and having motor activity, and the NH_2-terminal region is thought to be the site of the binding of cargoes (*55*) (Figs. 4 and 5).

In addition to intermediate and light intermediate chains, cytoplasmic dynein is associated with the protein complex dynactin (*56*). Dynactin contains 10 subunits: $p150^{Glued}$, $p135^{Glued}$ (a brain-specific variant of $p150^{Glued}$), p62, dynamitin (p50), actin-related protein 1 (Arp1), actin, actin-capping protein α subunit, actin-capping protein β subunit, p27, and p24, with a stoichiometry of 1:1:1:4:9:1:1:1:1:1. The $p150^{Glued}$-$p135^{Glued}$ heterodimer forms a side-arm projection from an Arp1-actin short filament (37 nm in length) and two small globular heads containing a microtubule binding site (*57*). The NH_2-terminal region of $p150^{Glued}$ forms a side arm that interacts with the 74-kD cytoplasmic dynein intermediate chain (*58*). Dynamitin probably links $p150^{Glued}$ to the Arp1-actin short filament, which possibly links cytoplasmic dynein to its cargo through $p150^{Glued}$. Thus, cytoplasmic dynein, especially its 74-kD intermediate chain, is probably linked to its cargo through the $p150^{Glued}$–Arp1-actin short filament complex, and the binding of $p150^{Glued}$ to Arp1 is mediated by dynamitin (*23*, *57*, *58*) (Fig. 5). Interestingly, $p150^{Glued}$ alone can bind to microtubules.

How does cytoplasmic dynein function in organelle transport? Immunocytochemical analysis, in vitro motility assays, antibody injection, and subcellular fractionation suggest that cytoplasmic dynein is a motor for the retrograde transport of membranous organelles in axons (*13*, *59*), the

distribution of late endosomes and lysosomes (60), the centrosomal localization of the Golgi complex (61), the vesicular transport from early to late endosomes (62), the apical transport of Golgi-derived membranes in intestinal epithelial cells (63), and the movement of phagosomes (64). Inhibition of the activity of dynactin by overexpressing dynamitin—which probably leads to the dissociation of cytoplasmic dynein from its cargoes—resulted in the dispersion of the Golgi complex, the redistribution of early and late endosomes toward the cell periphery, and suppression of the transport of intermediate compartment from the ER to the Golgi (65, 66) (Fig. 4).

All these functions could be related to members of the cytoplasmic dynein family. At present, several multiple cytoplasmic dynein heavy chains have been identified—at least two (CyDn and DLP4) in rat (20), two (DHC1A and DHC1B) in sea urchin (19), and three (DHC1, DHC2, and DHC3) (21) in HeLa cells. Conventional cytoplasmic dynein heavy chain (CyDn), DHC1A, and DHC1 are homologs and members of cytoplasmic dynein families; DLP4, DHC1B, and DHC2 are also homologs with each other. DHC2 is localized predominantly in the Golgi apparatus, whereas DHC3 is associated with as yet unidentified structures that may represent transport intermediates (21). Moreover, microinjection of antibodies to DHC2 causes dispersion of the Golgi complex (21). A recent study of cytoplasmic dynein gene knockout mice demonstrated that in $CyDn^{-/-}$ cells, the Golgi complex is fragmented and widely distributed in the cytoplasm, and late endosomes are distributed to the periphery of the cells (67) (Fig. 3). Thus, it is likely that both CyDn and DHC2 are involved in the formation and distribution of the Golgi complex. Because we do not have any data indicating the interaction of specific isoforms of the dynactin complex with specific members of the cytoplasmic dynein family, loss-of-function experiments with respect to specific members of the cytoplasmic dynein family need to be carried out to understand their individual roles in organelle transport.

Regulation of Bidirectional Transport

Regulation of bidirectional transport is an important mechanism in organelle transport. Because immunocytochemical analysis of ligated nerves indicates that the retrograde motor cytoplasmic dynein is conveyed to the cell periphery by anterogradely transported cargoes, a mechanism is required to dissociate anterograde and retrograde motors from membranous organelles at the cell periphery and to reassociate retrograde motors with retrogradely transported cargoes (59) (Fig. 3). The dissociation of anterograde and retrograde motors may involve the phosphorylation of kinesin and cytoplasmic dynein to regulate bidirectional transport (68). The activation and inactivation of motor proteins by phosphorylation and dephosphorylation may also be involved (68). It is also noteworthy that the direction of vesicle movement is regulated by the presence or absence of a tightly bound plus-end kinesin motor; that is, vesicles move retrogradely only when a retrograde motor is bound to the vesicles in the absence of an anterograde motor (69). In addition, the observation that guanosine 5′-O-(3′-triotriphosphate) (GTP-γ-S) inhibits vesicle transport in isolated squid axoplasm (70) suggests that small G proteins could be involved in the regulation of the association of motors to cargoes or the activation of the motors. More studies are necessary to clarify this issue.

Targeting of Motors

As outlined above, certain members of the kinesin and dynein superfamilies have been discovered, and each member has its own cargoes, although some redundancy may exist. Both kinesin and cytoplasmic dynein exhibit saturation binding to the vesicles, and proteolysis of vesicle membrane proteins abolishes binding (71). The COOH-terminal domain of KHC is thought to bind membranous cargoes, and this binding could be modulated by KLCs (32, 70, 72). However, it is currently unknown how a motor recognizes its own cargo.

As a candidate receptor for kinesin, kinectin was purified by kinesin affinity column chromatography (73). However, because kinectin also binds to cytoplasmic dynein and because it is plausible that cytoplasmic dynein binds to the cargo membranes through a dynactin-actin binding protein network (73), kinectin may not be a binding protein for kinesin in the cargo membranes, but may be a regulator of the binding of kinesin to the cargo. The identification of receptors for KIFs on cargoes is an important topic for future research. Cytoplasmic dynein may bind indirectly to cargoes through an interaction among the 74-kD intermediate chain, $p150^{Glued}$, dynamitin, and Arp1-actin short filament (Fig. 5). Because the Arp1-actin filament resembles the actin short filament in the spectrin-actin network underneath the erythrocyte plasma membrane, and because specific isoforms of ankyrin and spectrin associated with Golgi complex have been identified and because dynactin interacts with spectrin (74), it is possible that a receptor protein in the cargo membrane binds to the Arp1-actin filament and cytoplasmic dynein through the ankyrin-spectrin network (Fig. 5).

However, there are at least two or three members of the cytoplasmic dynein family that seem to bind to different organelles (20, 21). Although nothing is known about the isoforms of dynactin associating with new members of the cytoplasmic dynein family (because cytoplasmic dynein seems to be linked to its cargo indirectly through dynactin), the specificity of binding of cytoplasmic dynein with the cargo should be determined by the interaction of dynactin and receptors on the cargo. Although we have some understanding of how the motor binds to its own cargo, further studies are required to answer this intriguing question.

Conclusions and Future Challenges

A cell uses a surprisingly large number of microtubule-associated motor proteins (more than 20 KIFs and at least two or three

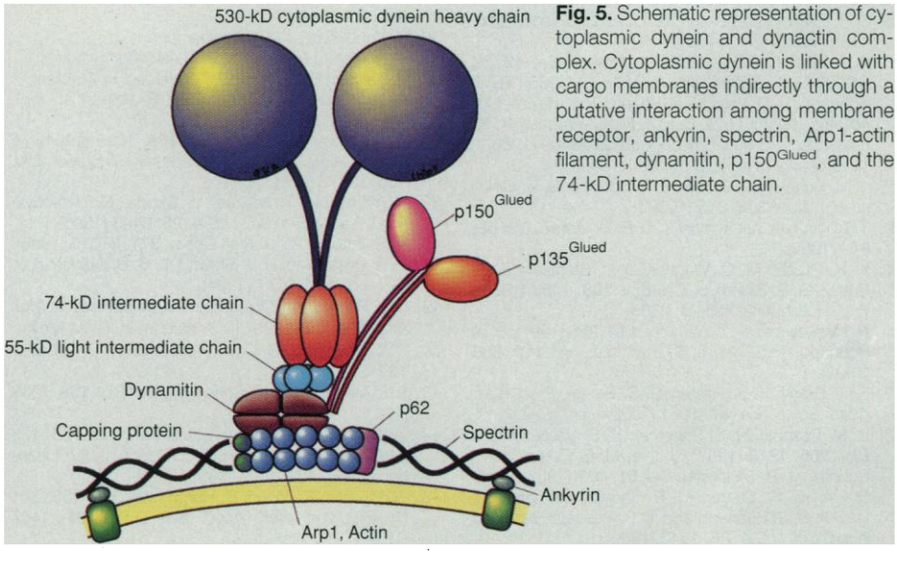


Fig. 5. Schematic representation of cytoplasmic dynein and dynactin complex. Cytoplasmic dynein is linked with cargo membranes indirectly through a putative interaction among membrane receptor, ankyrin, spectrin, Arp1-actin filament, dynamitin, $p150^{Glued}$, and the 74-kD intermediate chain.

cytoplasmic dynein family members have been identified), which precisely control the direction and velocity of transport of various kinds of cargoes. These cargoes include distinct types of membranous organelles, possibly protein complexes and mRNA (*1*, *43–45*, *75*, *76*). The control of transport involves developmental regulation as well. Because important motors still remain to be identified, we need to search for them. Molecular cell biological and molecular genetics approaches may enable the characterization of newly identified members, the analyses of the functions of each member, and the biological significance of the transports performed by each member. Understanding the mechanism of the recognition of the correct cargo by each motor is also important. How and where the cargoes bind to their correct motors, and how the cargoes are dissociated from the motors at their destination, are also intriguing topics for future research. The regulation mechanisms may involve phosphorylation and dephosphorylation of motors and receptors on the cargoes, or may involve small G proteins and signal transduction cascades. Organelle transport and membrane traffic are deeply related to each other. In the near future, we will fully understand how the cell transports and sorts proteins and lipids to their appropriate destinations in order to build itself. We are beginning to obtain abundant new information regarding the mechanism of organelle transport in cells, while at the same time fascinating new questions are arising that will lead to exciting research in this field.

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