Kinesin Family in Murine Central Nervous System

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Abstract. In neuronal axons, various kinds of membranous components are transported along microtubules bidirectionally. However, only two kinds of mechanochemical motor proteins, kinesin and brain dynein, had been identified as transporters of membranous organelles in mammalian neurons. Recently, a series of genes that encode proteins closely related to kinesin heavy chain were identified in several organisms including Schizosaccharomyces pombe, Aspergillus niddulans, Saccharomyces cerevisiae, Caenorhabditis elegans, and Drosophila. Most of these members of the kinesin family are implicated in mechanisms of mitosis or meiosis. To address the mechanism of intracellular organelle transport at a molecular level, we have cloned and characterized five different members (KIFI-5), that encode the microtubule-associated motor domain homologous to kinesin heavy chain, in murine brain tissue. Homology analysis of amino acid sequence indicated that KIFI and KIF5 are murine counterparts of unc104 and kinesin heavy chain, respectively, while KIF2, KIF3, and KIF4 are as yet unidentified new species. Complete amino acid sequence of KIF3 revealed that KIF3 consists of NH2-terminal motor domain, central α-helical rod domain, and COOH-terminal globular domain. Complete amino acid sequence of KIF2 revealed that KIF2 consists of NH2-terminal globular domain, central motor domain, and COOH-terminal α-helical rod domain. This is the first identification of the kinesin-related protein which has its motor domain at the central part in its primary structure. Northern blot analysis revealed that KIFI, KIF3, and KIF5 are expressed almost exclusively in murine brain, whereas KIF2 and KIF4 are expressed in brain as well as in other tissues. All these members of the kinesin family are expressed in the same type of neurons, and thus each one of them may transport its specific organelle in the murine central nervous system.

A neuron consists of a cell body and long processes, i.e., a single long axon and dendrites. Various intracellular components are synthesized within the cell body, some of which are transported in the axon to their sites of utilization at two separate flow rates, fast and slow (Grafstein and Forman, 1980). Membranous organelles are mainly transported by the fast flow, while proteins such as cytoskeletal elements are conveyed by the slow flow. Electron microscopic studies on axons in vivo have identified crossbridge structures between microtubules and membranous organelles as candidates for membranous organelle translocators (Hirokawa, 1982; Miller and Lasek, 1985; Hirokawa and Yorifuji, 1986; Hirokawa et al., 1989). Recently, two kinds of proteins, kinesin and cytoplasmic dynein, have been identified in neurons as microtubule-dependent motors which generate the force necessary for the fast axonal transport of membranous organelles (Vale et al., 1985a,b; Brady, 1985; Lye et al., 1987; Paschal and Vallee, 1987). Because kinesin moves to the plus end of microtubules (Vale et al., 1985b; Porter et al., 1987) and because it is associated primarily with anterogradely moving membranous organelles in the axon (Hirokawa et al., 1991), it is considered to be an anterograde translocator. On the other hand, brain dynein moves toward the minus end of microtubules, and is therefore a candidate for a retrograde transporter in the axon (Paschal and Vallee, 1987; Schroer et al., 1989; Schnapp and Reese, 1989; Hirokawa et al., 1990).

Kinesin consists of two heavy chains and several light chains (Kuznetsov et al., 1988; Bloom et al., 1988; Murofushi et al., 1988; Hirokawa et al., 1989). Kinesin heavy chain (KHC) consists of an amino-terminal globular motor domain which generates force for movement on microtubules by ATP hydrolysis, a central α-helical coiled-coil stalk domain, and a carboxy-terminal fan-like domain which interacts with light chains and possibly with cargoes (Hirokawa et al., 1989; Yang et al., 1989; Scholey et al., 1989).

Recently a series of genes that encodes proteins closely related to KHC were identified in several organisms including Schizosaccharomyces pombe (cut7; Hagan and Yanagida, 1990), Aspergillus niddulans (bimC; Enos and Morris, 1990), with...
Drosophila (ncd; Saccharomyces cerevisiae (KAR3; Meluh and Rose, 1990), Caenorhabditis elegans and unordered; Otsuka et al., 1991), and Drosophila (endow et al., 1990; McDonald and Goldstein, 1990) (nod; Zhang et al., 1990). Most of these proteins are implicated in mechanisms of mitosis or meiosis. It was also reported that Drosophila contains at least six additional kinesin proteins (KLP6-6; Stewart et al., 1991), and that the kinesin family consists of about 35 members in Drosophila (Endow and Hatsumi, 1991). All the members share a homologous domain of ~350 amino acids which contains a putative ATP binding site and a microtubule-binding site. Therefore, these proteins are likely to be microtubule-associated motors. Since the nonmotor regions of the kinesin family members show few apparent similarities, these proteins are considered to be kinesin homologues with divergent functions that have arisen due to gene duplication. Because the nonhomologous domain of KHC includes a region bound to vesicles, the nonhomologous region of the kinesin family members is also likely to contain a site(s) which allows each motor to recognize a specific cargo.

In nerve cells, some vesicles are transported in the dendrites while others are transported into the axon. Even in the axon, various kinds of intracellular components (such as mitochondria, precursors of synaptic vesicles, precursors of axonal plasma membranes, dense-cored vesicles, etc.) are transported along microtubules and stationed at specific positions. It seems impossible that only a single unidirectional motor protein would transport various kinds of components correctly to their target points. Electron microscopic observations have suggested that the detailed structures of cross-bridges between microtubules and different kinds of membranous organelles are distinct (see Hirokawa, 1982; Hirokawa et al., 1985, 1989; Hirokawa and Yorifuji, 1986). Therefore we speculated that several other, new members of the kinesin family may exist within mammalian neuronal cells. This speculation is also supported by the fact that a possible new translocator responsible for the transport of synaptic vesicles was recently identified in C. elegans (Hall and Hodgcock, 1991).

In this report we describe the identification and characterization of five members of kinesin family (KIF1-5) in the murine central nervous system (CNS). This is the first report covering genes of the mammalian kinesin family. The regions used correspond to amino acids 88-94 (IFAYGQ) and 238-243 (LAIS$E) of kinesin heavy chain (see Fig. 1). A 5' primer was used in PCR with each of four different 3' primers. The 5' primer was:

A: AT(A,C,T)TT(C,T)GC(A,C,G,T)TA(C,T)GG(A,C,G,T)TC(A,G)AC

The 3' primers were:

B1: TC(A,C,G,T)AA(A,G,C,T)CC(A,G,C,T)GC(A,G,C,T)AG(A,G)TC
B2: TC(A,G,C,T)AA(A,C,G,T)CC(A,G,C,T)CC(A,G,C,T)AA(A,C,G,T)
B3: TC(A,G,C,T)AA(A,C,G,T)CC(A,G,C,T)GC(A,G,C,T)AG(A,G)TC
B4: TC(A,G,C,T)AA(A,C,G,T)CC(A,G,C,T)GC(A,G,C,T)AA(A,C,G,T)

An aliquot of each PCR was analyzed by agarose gel electrophoresis. The amplified DNAs at a size of ~450 bp were eluted from the gel, treated with the Klenow fragment of DNA polymerase I (Toyobo), and then phosphorylated with T4 polynucleotide kinase (Toyobo). The products were then cloned into the HinclII site of pUC18 (Takara, Tokyo, Japan), which had been dephosphorylated using bacterial alkaline phosphatase (Takara). To classify the clones containing inserts into groups, we constructed sequencing reactions of 84 of them. One member of each group (pKIFI, pKIF2, pKIF3, pKIF4, and pKIF5) was then fully sequenced by the dideoxy chain-termination method (Sanger et al., 1977).

Isolation of cDNA Clones

cDNA clones were isolated by standard methods from the above-mentioned murine brain library. $P$-labeled probes for KIF2 and KIF3 were generated as described above. The isolated recombinant plasmid DNA insert was subcloned into the plasmid vector pUC18. After further subcloning, both strands of the cDNA inserts were sequenced.

Northern Blot Analysis

Total RNA and poly(A) RNA were prepared from murine tissues as described above. RNA was quantified by measuring the absorbance at 260 nm, and the integrity of the RNA was checked by staining after agarose gel electrophoresis. The RNA sample was denatured and electrophoresed in agarose gel containing 2.2 M formaldehyde. After electrophoresis, the RNA was transferred to a nylon filter (HybondTM-N+, Amersham Corp., Arlington Heights, IL). As a hybridization probe, we used each cDNA insert of pKIF5-5 using BamHI and PstI for KIF1, 3, 4, and 5, and BamHI and HindIII for KIF2. The probes were labeled by a multiprime DNA labeling system. Hybridization was carried out at 65°C for 8 h in a rapid hybridization buffer (Amersham Corp.). The filters were washed in 0.1 X SSC, 0.1% SDS at 65°C for 30 min and exposed to X-ray film.

In Situ Hybridization

In situ hybridization histochemistry was performed as described previously (Takemura et al., 1991) with some modifications. Briefly, adult mice were fixed by perfusion with cold 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. The brains were then removed and fixed in the same buffer for an additional 2 h at 4°C. They were then cryoprotected overnight in 20% sucrose in 0.1 M phosphate buffer containing 0.2% paraformaldehyde, frozen, and cryostat sections (16 µm thick) were cut and mounted on gelatin-coated slides. The sections were dried, treated with 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl, pH 8 for 10 min, and immersed in a prehybridization buffer solution (4X SSC, 1X Denhardt's solution) for 35 min. They were then dehydrated in ethanol, and hybridized overnight at 42°C in a humidified chamber with 32P-labeled cDNA probe suspended in 350 µl of hybridization buffer (50% deionized formamide, 4X SSC, 0.12 M sodium phosphate, pH 7.2, 1% sarcosyl, 1X Denhardt's solution, 0.5 ng/ml.
sonicated salmon sperm DNA, 20 mM DTT). The sections were then rinsed briefly in 4× SSC at room temperature and then at 55°C and to a final stringency of 0.2× SSC at 55°C for 15 min in the presence of 20 mM 2-mercaptoethanol, and washed extensively in 1× SSC at room temperature and then at 55°C and to a final stringency of 0.2× SSC at 55°C for 15 min in the presence of 20 mM 2-mercaptoethanol, and washed.

Results

Identification of the Kinesin Family in Murine CNS

The motor domain of the kinesin heavy chain contains two amino acid sequences conserved almost completely among all known kinesin family members. One pair of degenerate primers corresponding to the conserved sequences (primers A and B, Fig. 1) was used in the PCR experiment to amplify segments of cDNAs encoding the motor domain of kinesin family from the murine brain cDNA library. Since primer B is too highly degenerated for PCR, we used each of four subprimers (primers B1-4) in the PCR experiment with primer A (see Materials and Methods). Amplification of murine brain cDNA with primers A and B, Fig. 1) was used in the PCR experiment to amplify the remaining 64 clones were not related to kinesin. The PCR fragments encoding the kinesin family fell into five classes. Each class encoded a predicted protein that was homologous to KHC in the region between primers A and B (Fig. 1). We have designated these proteins as KIF1-5. KIF1 is 73.6% identical in amino acid sequence to a predicted protein encoded by C. elegans unc104 gene between the two used primers, and thus KIF1 is considered to be a murine counterpart of unc104 (Table I). Between primers A and B, KIF5 is 80.1, 83.3, 84.6, and 89.1% related to kinesin. The PCR fragments encoding the kinesin family were divided by the total numbers of compared residues. Values of >70% are indicated by sequence analysis. The remaining 64 clones were not related to kinesin. The PCR fragments encoding the kinesin family fell into five classes. Each class encoded a predicted protein that was homologous to KHC in the region between primers A and B (Fig. 1). We have designated these proteins as KIF1-5. KIF1 is 73.6% identical in amino acid sequence to a predicted protein encoded by C. elegans unc104 gene between the two used primers, and thus KIF1 is considered to be a murine counterpart of unc104 (Table I). Between primers A and B, KIF5 is 80.1, 83.3, 84.6, and 89.1% identical in amino acid sequence to the Drosophila KHC (Yang et al., 1989), sea urchin KHC (Wright et al., 1991), squid KHC (Kosik et al., 1990), and human KHC (Navone et al., 1992), respectively. Thus KIF5 is considered to be a murine counterpart of KHC. KIF2-4 show less than 50% identity to the previously known eight kinesin members.

Table I. Homology (Percent Identity) of Motor Domains of KIF1-5 for Those of the Previously Reported Kinesin Family

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their PCR fragments. To examine the detailed structures of KIF2 and KIF3, we determined the complete nucleotide and predicted protein sequences. We screened KIF2 cDNA from nod, and bimC) comparison of KIF2 and KHC demonstrated that KIF2 has acid residues (Mr, 80,945; Fig. 2). Diagonal dot matrix the 5-d-old murine brain cDNA library using the PCR- KIF2 and KIF3, we determined the complete nucleotide and sequence analysis revealed the presence of a single open reading frame of 2,148 nucleotides that encoded 716 amino residues.

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Figure 2. Nucleotide and predicted amino acid sequence of KIF2. The nucleotide residues are numbered from the first letter of the initiation codon. The initiation methionine codon follows an in-frame termination codon (boxed). The putative ATP-binding site in the motor domain is underlined. The TAA stop codon is marked with an asterisk. These sequence data are available from EMBL/DDBJ under accession number D12644.
tides that encoded 701 amino acid residues (Mr, 80,167; predicted to construct a globular structure. The carboxy-terminal domain (amino acid residues 587-701) is hydrophilic and to make mainly α-helical struct-

bility of the KIF3 product to other kinesin-related proteins is decreasingly so in adult murine brains, suggesting that KIF3 may be developmentally expressed and, tissue distribution of each of the KIF1-5 transcripts (Figs. 5 and 6). We used the PCR-amplified cDNA fragments of KIF1-5 as the probes. Each probe reacted with its specific transcript and no cross-reaction among KIFI-5 was detected under the conditions used. A major transcript of KIF1 is 6 kbp (Fig. 5), similar to the reported size of the KHC mRNA. We can also detect a trace amount of KIF3 transcript was detected in various tis-

Northern blot analysis detected three KIF3 transcripts of 4.5, 3.5, and 2.5 kbp in length (see Fig. 5). So, our KIF3 cDNA clones are thought to correspond to the 2.5-kbp transcript. Diagonal dot matrix comparison of KIF3 and KHC demonstrated that KIF3 has an amino-terminal domain with a unique carboxy-terminal tail domain (Fig. 4). The KIF3 transcript was detected in all adult tissues examined (Fig. 6).

Two minor transcripts of 4.5 and 3 kbp were also detected. These transcripts were expressed in 5-d-old murine brain but not in adult murine brains. We found three kinds of transcripts of KIF3 at sizes of 4.5, 3.5, and 2.5 kbp (Fig. 5). The three transcripts were all expressed at an equal level in both 5-d-old and adult murine brains. We re-

Northern blot analysis was performed to study the size, de-

Developmental Expression and Tissue Distribution of KIFI-5 mRNAs

Northern blot analysis was performed to study the size, developmental expression, and tissue distribution of each of the KIF1-5 transcripts (Figs. 5 and 6). We used the PCR-amplified cDNA fragments of KIF1-5 as the probes. Each probe reacted with its specific transcript and no cross-reaction among KIFI-5 was detected under the conditions used. A major transcript of KIF1 is 6 kbp (Fig. 5), similar to the reported size of the KHC mRNA. We can also detect a trace amount of KIF3 transcript was detected in various tis-

Figure 3. Nucleotide and predicted amino acid sequence of KIF3. The nucleotide residues are numbered beginning at the first letter of the initiation codon. The initiation methionine codon follows an in-frame termination codon (boxed). The putative ATP-binding site in the motor domain is underlined. The TAA stop codon is marked with an asterisk. The polyadenylation signals are indicated by the dashed lines below the sequence. These sequence data are available from EMBL/DDBJ under accession number D12645.
Figure 4. Diagonal dot matrix comparison between KHC and KIF2 (a) or KIF3 (b). Comparisons were performed using a SDC-GENETYX computer program. The dots correspond to midpoints of 15-residue spans, giving a double-matching probability that the mean score is ≥ 2.0.

A major transcript of KIF4 was 4 kbp, and was expressed in 5-d-old murine brain but not in the adult one (Fig. 5). The KIF4 transcript was predominantly expressed in spleen among adult tissues (Fig. 6).

A transcript of KIF5 (murine KHC) was 5 kbp, and is expressed in both 5-d-old and adult murine brains (Fig. 5). Surprisingly, we found that the transcript of KHC was expressed almost exclusively in brain tissue and was not detected in the other examined tissues (Fig. 6), although KHC was reported to exist ubiquitously by biochemical and immunological techniques.

In Situ Hybridization of KIFs in CNS

Since no cross-reaction was detected among probes used in Northern blot analysis, we used exactly the same probes for in situ hybridization analysis. KIF1, KIF2, and KIF5 transcripts were expressed in brain gray matter, and were particularly abundant in the hippocampus, cerebral cortex, and cerebellar cortex (Fig. 7, A–D, G, and H). KIF3 mRNA was also expressed in both cerebral and cerebellar gray matter, but the signal in cerebrum was much weaker than that in the cerebellum (Fig. 7, E and F). In the cerebellum, the signals of KIF1, KIF2, KIF3, and KIF5 were intense in the granular cell layer which includes cell bodies of Purkinje cells and granular cells (Fig. 8, A, C, E, and G). In the hippocampus, the CA3 region had the highest level of the KIF3 and KIF5 transcripts while KIF1 and KIF2 were expressed in CA1, CA3, and dentate gyrus (Fig. 8, B, D, F, and H). Observations with higher magnification revealed that signals of all the examined KIFs in the hippocampus were detected on pyramidal cells in the hippocampus (Fig. 9) and also in the Purkinje cells and granule cells in the cerebellum (data not shown). This result provides evidence that KIF1, KIF2, KIF3, and KIF5 are expressed in neurons, and that all the examined KIFs are expressed in a single neuronal cell.

Discussion

Until now, only two kinds of microtubule-associated motor proteins, kinesin and brain dynein, have been identified in the mammalian CNS. Although several kinesin-related genes were recently identified in various organisms, they all participate in cell division, with the only exception being uncl04, which is considered to play a role in axonal transport in C. elegans. In this study, we demonstrated for the first
time that at least five kinesin-related genes (KIF1-5) are expressed in mammalian CNS. All KIF1-5 share a homologous domain which include a putative ATP-binding site and a microtubule-binding site of KHC. It was clearly demonstrated that the conserved motor domain of KHC is both necessary and sufficient to move along microtubules in the presence of ATP in vitro (Yang et al., 1990). Taking these data together, we concluded that murine CNS contains at least six microtubule-associated motor proteins, that is, five kinesin-related proteins (KIF1-5) and a brain dynein.

We demonstrated that the expression pattern of the kinesin family is regulated by developmental stage and cell types. KIF1, KIF3, and KIF5 are expressed almost exclusively in the brain tissue in adult mice. Of the three, the KIF3 transcript is expressed mainly in the cerebellar granular layer while the other two are expressed both in the cerebellum and cerebrum. On the other hand, KIF2 is expressed among various tissues ubiquitously, and its expression level in brain decreases with development. KIF4 is expressed in adult murine spleen and juvenile murine brain, but its expression is almost completely absent from the adult murine brain. These complex regulations of the expression of KIFs suggest that each member of the KIFs has its special function. For example, each KIF may transport its own specific cargo.

Figure 7. Expression pattern of KIF1, KIF2, KIF3, and KIF5 in adult murine cerebrum and cerebellum. Transverse sections of adult murine cerebrum (A, C, E, and G) and sagittal sections of cerebellum (B, D, F, and H) were stained with cloned PCR fragments of KIF1 (A and B), KIF2 (C and D), KIF3 (E and F), and KIF5 (G and H). Slides were exposed to Kodak X-OMAT AR film at -80°C for 1 wk. c, cerebral cortex; and h, hippocampus. Bar, 0.5 μm.

Sequence homology indicated that KIF1 is the murine counterpart of C. elegans unc-04. The phenotype of unc-04 mutants demonstrated the abnormal accumulation of synaptic vesicles in cell bodies and the loss of some neuromuscular junctions (Hall and Hedgecock, 1991). Thus, unc-04 is considered to play a role in the fast axonal transport of synaptic vesicles in C. elegans. However, the structure of nerve cells in C. elegans is very different from that of nerve cells in higher vertebrates, and possibly the way to supply synaptic vesicles to the nerve terminals could also be different. Therefore, it remains to be determined what the function of KIF1 in mammalian nerve cells is.

KIF3 is almost exclusively expressed in brain tissue, and consists of an NH2-terminal globular motor domain, a central α-helical coiled-coil stalk domain and a COOH-terminal globular domain. This nature of KIF3 is similar to those of unc-04 and KHC which play a role in the vesicle transport in neurites. The protein sequence of KIF3 shows a significant similarity to the recently reported partial amino acid sequence of Drosophila KLP4 (Stewart et al., 1991). But the KLP4 transcript was shown to be expressed in non-neuronal tissues as well as Drosophila heads, and thus at present we cannot clearly state whether KLP4 is a Drosophila counterpart of KIF3 or not.

KIF2 and KIF4 exist not only in brain but also in other tissues. KIF2 shows a “central motor” type of the kinesin family, which is quite distinct from the other members. At present we still have no idea about the specific functions of KIF2 and KIF4, but information on their localization in cells, motor activity in vitro, molecular shape, and phenotype of transgenic mice will give us some insight into their in vivo function.

We demonstrated that the KIF5 (KHC) transcript was expressed almost exclusively in the brain. This observation is quite consistent with the previous report that Drosophila KHC mutants are considered to get damaged in their neural function especially in the neuromuscular system (Saxton et al., 1991). Our immunocytochemical study of ligated nerve axons also strongly suggested that kinesin plays an important role in the anterograde membrane transport (Hirokawa et al., 1991). On the other hand, kinesin was identified from non-neuronal sources, such as sea urchin eggs (Scholey et al., 1985), Drosophila embryo (Saxton et al., 1988), bovine adrenal medulla (Murofushi et al., 1988), and various cultured cell lines (Neighbors et al., 1988; Pfister et al., 1989) by cosedimentation method with microtubules in the presence of AMP-PNP or by immunological techniques. This is inconsistent with our data of the neuron-specific expression of KIF5. Two possible explanations can be put forward: (a) the protein which was identified as KHC in non-neuronal sources is not KHC itself but one single member of the kinesin family; and (b) KHC transcript is expressed ubiquitously in various tissues, but its expression level in non-neuronal
Figure 8. Expression pattern of KIF1, KIF2, KIF3, and KIF5 in a single lobe of adult murine cerebellum and hippocampus of adult murine cerebrum. Sagittal sections of a single lobe of cerebellum (A, C, E, and G) and transverse sections of hippocampus (B, D, F, and H) were stained with cloned PCR fragments of KIF1 (A and B), KIF2 (C and D), KIF3 (E and F), and KIF5 (G and H). The same slides in Fig. 7 were further processed by dipping in 1:1 Ilford K-5 emulsion, and exposed for 1-2 wk. The developed slides were observed with an Axiophoto microscope using dark-field systems, so the silver grains appear as white spots. CA1 and CA3, CA1 and CA3 areas of the hippocampus; W, cerebellar white matter; IGL, cerebellar inner granular layer; and ML, molecular layer. Bar, 100 µm.
Figure 9. Expression pattern of KIF1, KIF2, KIF3, and KIF5 in the murine hippocampus. Transverse sections of adult murine cerebrum were stained with cloned PCR fragments of KIF1 (A), KIF2 (B), KIF3 (C), and KIF5 (D). The slides were further processed by dipping in 1:1 Ilford K-5 emulsion, and exposed for 1-2 wk. The developed slides were observed by an Axiophoto microscope using bright-field systems, so the silver grains appear as black spots. Silver grains are observed on the pyramidal cells in all experiments. Bar, 10 μm.

The expression level of KHC is too low to be detected by our Northern blot analysis. We amplified the kinesin family by PCR with primers A and B using the cDNA library of a bovine kidney cell line (MDBK) as a template, and identified the amplified fragments of KHC (data not shown). This result supports the later possibility. It should be also mentioned that several kinesin-related proteins have been identified very recently by immunological approaches in sea urchin eggs and *Xenopus* eggs (Cole et al., 1992; Sawin et al., 1992). The next question would be whether KHC transports the same cargo in the axon and in non-neuronal cells or not.

Why were KIFI-4 not identified until now by cosedimentation analysis with microtubules in the presence of AMP-PNP from mammalian brains? One possible explanation is that KIF1-4 are not cosedimented with microtubules even in the presence of AMP-PNP. But this is not likely because KIFI-4 share a motor domain homologous to that of KHC. Another possibility is that KIF1-4 are bound so tightly to membranous components that the motors are insoluble in the commonly used extraction buffer without detergents. Yet another possibility is that the quantity of KIF1-4 is much smaller than that of KHC, despite the fact that the expression levels of KIFI-4 transcripts are comparable to that of KHC (Fig. 2).

Various kinds of membranous components are transported
in neurites anterogradely. Some are transported from the cell body to the synapse through the axon, some into the axon itself, and others from cell body to dendrites. In addition to the membranous organelles, slow components (cytoskeletal proteins such as actin, neurofilament triplet proteins, tubulin, etc.) are also transported by unknown motors. Exactly how does the neuronal cell regulate the transport along microtubules and the positioning of various intracellular components? The first step of the transport is considered to be the recognition of the cargo by its specific transporter. Since KIF1-5 contain a unique tail region outside of their common motor domain, they may recognize their specific cargo. We also demonstrated that all of the KIF1-5 exist in the cerebellum. Therefore, the kinesin family may recognize, transport, and position their specific cargoes in a single neuronal cell. Future studies on biochemical and physicochemical characterization, molecular structure and intracellular localization will give us clear answers about the functions of KIF1-5.

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