Two Kinesin Light Chain Genes in Mice

IDENTIFICATION AND CHARACTERIZATION OF THE ENCODED PROTEINS

(Received for publication, January 8, 1998, and in revised form, April 2, 1998)

Amena Rahman‡, Dara S. Friedman‡, and Lawrence S. B. Goldstein‡‡

From the ‡Howard Hughes Medical Institute, Division of Cellular and Molecular Medicine, Program in Biomedical Sciences and Department of Pharmacology, University of California San Diego, La Jolla, California 92039-0683 and the ‡Department of Pharmacology and Biochemistry, University of California, San Francisco, California 94143

Native kinesin consists of two light chains and two heavy chains in a 1:1 stoichiometric ratio. To date, only one gene for kinesin light chain has been characterized, while a second gene was identified in a genomic sequencing study but not analyzed biochemically. Here we describe new genes encoding kinesin light chains in mouse. One of these light chains is neuronally enriched, while another shows ubiquitous expression. The presence of multiple kinesin light chain genes in mice is especially interesting, since there are two kinesin heavy chain genes in humans (Niclas, J., Navone, F., Hom-Booher, N., and Vale, R. D. (1994) Neuron 12, 1059–1072). To assess the selectivity of kinesin light chain interaction with the heavy chains, we performed immunoprecipitation experiments. The data suggested that the light chains form homodimers with no specificity in their interaction with the two heavy chains. Immunofluorescence and biochemical subfractionation suggested differences in the subcellular localization of the two kinesin light chain gene products. Although both kinesin light chains are distributed throughout the central and peripheral nervous systems, there is enrichment of one in sciatic nerve axons, while the other shows elevated levels in olfactory bulb glomeruli. These results indicate that the mammalian nervous system contains multiple kinesin light chain gene products with potentially distinct functions.

Kinesin is a molecular motor that generates ATP-dependent movements along microtubules (2, 3) of vesicles and organelles (4–8). Efficient transport is particularly important in neurons where vesicles and organelles move substantial distances from sites of synthesis in the cell body to sites of activity at the axonal termini. Loss of kinesin heavy chain expression in Drosophila (9), Caenorhabditis elegans (10), and mammalian cultured cells (11, 12) produced phenotypes consistent with a role for kinesin in axonal transport.

Native kinesin is a tetramer of two kinesin heavy chains (KHC) of approximately 120 kDa and two kinesin light chains (KLC) of 60–70 kDa (13, 14). KHC contains three distinct domains: an N-terminal motor containing the ATP and microtubule binding sites (15), an α-helical coiled-coil believed to be involved in dimerization (16), and a globular C-terminal tail. The tail and parts of the α-helical coiled-coil are thought to be involved in binding KLC (17, 18) and vesicular cargo (19, 20). KHC alone is capable of ATP-dependent movement along microtubules in vitro (21). The role of KLC in the native complex is poorly understood. Notably, KLC structure is highly conserved. Sequences that are most conserved include an N-terminal coiled-coil region of 107 amino acids and six modular imperfect repeats of 34 amino acids known as tetra-trico peptide repeats (TPR) encompassing a total of 252 amino acids (22). The KLC coiled-coil domain is necessary and sufficient for the interaction with KHC (17). The TPR domain has been implicated in protein-protein interaction in a large group of structurally and functionally diverse proteins (23). Thus, it is probable that the KLC TPR domain is involved in either direct binding to other proteins, e.g. cargo, or in interactions with KHC. Strikingly, KLC mRNA is alternatively spliced in rat, squid, sea urchin, and worms (24–27). It has been suggested that functional diversity of the kinesin complex arises from the ability of KLC to form various isofoms.

Although KLC genes have been cloned from several organisms (rat (24), Drosophila (17), squid (25), sea urchin (26), C. elegans (27), and humans (28), only one gene has been identified within any nonmammalian species. In this study, we use sequence analysis and genetic mapping to demonstrate that KLC is encoded by multiple genes in mice. The data show that the expression and behavior of two of the KLC gene products are different. Together, our data suggest that the two characterized kinesin light chains and the four separate homologs produced by the known patterns of association have distinct functions.

EXPERIMENTAL PROCEDURES

Cloning and Sequencing—Mouse KLC genes were identified by PCR using degenerate oligonucleotides in the TPR domain. The nucleotide sequences for the coding and noncoding primers were GGCCCGAGCTCGT/G/AT/F/G/C/A/AA/C/T/A/ATG/C/T/TC/G/A/AA/C/T/AT and CGCGGTACCTGT/G/T/CC/C/T/TG/TA/AA/G/A/TT/G/T/TGG/C/A/G/A/G, respectively. Cloning of SaeI and KpnI restriction sites allowed for directed cloning of PCR products into pBluescript (SK+) plasmid (Stratagene). The source of DNA for PCR amplification was a Balb/c neonatal mouse brain cDNA library in a Zap (Stratagene). PCR conditions were as follows: denaturation at 94 °C for 10 min; 5 cycles of denaturation at 94 °C for 45 s, annealing at 42 °C for 1 min, 1-min ramp to 72 °C, and elongation at 72 °C for 1 min; 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min; final elongation at 72 °C for 10 min; and a final cooling to 4 °C. Two different 300-bp PCR amplification products were obtained that resembled the known rat KLC sequence (24). These 300-bp fragments of heavy chain; uKHC, ubiquitous kinesin heavy chain.
KLC1 and KLC2 were used to probe the same cDNA library for full-length clones by standard methods (29). The longest clone from each screening was sequenced on both strands (Sequenase kit; U.S. Biochemical Corp.). Complete sequence of KLC2 was obtained by 5'-RACE (5'-RACE system for rapid amplification of cDNA ends, version 2.0; Gibco Life Technologies, Inc.) and 5' rapid amplification of cDNA ends (RACE) was performed by the manufacturer, and subcloned into pGEX-KG (31) to produce a glutathione S-transferase (GST)-tagged fusion protein. An affinity-purification column was loaded with 0.2 mM isopropyl-1-thio-β-D-galactoside (140 mM NaCl, 2.5 mM KCl, 10 mM dibasic sodium phosphate, 2 mM monobasic potassium phosphate, pH 7.4) and washed 3 times with 0.1% SDS at 65 °C. Polymorphism patterns were sent to Jackson Laboratory for analysis; chromosomal localization was assigned relative to other genes previously mapped by Jackson Laboratory.

**Northern Analysis**—Total RNA was made from mouse tissues using Trizol (Gibco Life Technologies, Inc.) as per the manufacturer's instructions. RNA was fractionated by electrophoresis (32) on 7.5% SDS-polyacrylamide gels and transferred by the manufacturer. Autoradiography was done as described in their protocols.

**Antibody Production**—An NcoI/Xhol fragment of KLC1, which accounts for all but the first seven amino acids of the sequence, was subcloned into pGEX-KG (31) to produce a glutathione S-transferase (GST)-tagged fusion protein. An affinity-purification column was loaded with 0.2 mM isopropyl-1-thio-β-D-galactoside (140 mM NaCl, 2.5 mM KCl, 10 mM dibasic sodium phosphate, 2 mM monobasic potassium phosphate, pH 7.4) and washed 3 times with 0.1% SDS at 65 °C. Polymorphism patterns were sent to Jackson Laboratory for analysis; chromosomal localization was assigned relative to other genes previously mapped by Jackson Laboratory.

**Antibody Production**—An NcoI/Xhol fragment of KLC1, which accounts for all but the first seven amino acids of the sequence, was subcloned into pGEX-KG (31) to produce a glutathione S-transferase (GST)-tagged fusion protein. An affinity-purification column was loaded with 0.2 mM isopropyl-1-thio-β-D-galactoside (140 mM NaCl, 2.5 mM KCl, 10 mM dibasic sodium phosphate, 2 mM monobasic potassium phosphate, pH 7.4) and washed 3 times with 0.1% SDS at 65 °C. Polymorphism patterns were sent to Jackson Laboratory for analysis; chromosomal localization was assigned relative to other genes previously mapped by Jackson Laboratory.

**Antibody Production**—An NcoI/Xhol fragment of KLC1, which accounts for all but the first seven amino acids of the sequence, was subcloned into pGEX-KG (31) to produce a glutathione S-transferase (GST)-tagged fusion protein. An affinity-purification column was loaded with 0.2 mM isopropyl-1-thio-β-D-galactoside (140 mM NaCl, 2.5 mM KCl, 10 mM dibasic sodium phosphate, 2 mM monobasic potassium phosphate, pH 7.4) and washed 3 times with 0.1% SDS at 65 °C. Polymorphism patterns were sent to Jackson Laboratory for analysis; chromosomal localization was assigned relative to other genes previously mapped by Jackson Laboratory.
mouse brain cDNA library. Two 300-bp products with similar but not identical sequences were obtained. These two products were used as probes to screen for longer clones from the same library. The longest cDNA clone of each of the mouse KLC products was completely sequenced on both strands. One of the clones, KLC1, yielded a full-length sequence of 2289 bp (GenBank accession number AF055665) and encoded both the translation start site and the poly(A) tail. The other clone, KLC2, was 2682 bp in length and contained the poly(A) tail but not the translation start site. The 5'-end of KLC2 was obtained by 5'-RACE and directly sequenced to finish KLC2 full-length sequence (GenBank accession number AF055666).

The two mouse kinesin light chain sequences were compared with each other using the Genetics Computer Group GAP program (30). Mouse KLC1 and KLC2 predicted polypeptide sequences were almost identical in the 34-amino acid modular TPR domains (Fig. 1A) and also showed a high degree of similarity in the N-terminal coiled-coil region. The linker region between the coiled-coil and the TPR domain and the C-terminal ends diverged the most between the two mouse KLCs. KLC1 and KLC2 were predicted to form protein products of 61 and 67 kDa, respectively.

Multiple sequence alignments of KLC1, KLC2, and kinesin light chains cloned from various organisms suggest that there are three classes of KLC genes (Fig. 1B). Kinesin light chain sequences from sea urchin, squid, Drosophila, rat, and human KLC seem to be true homologues of mouse KLC1. A third kinesin light chain sequence (referred to as KLC3 throughout this paper) in humans and mice was recently identified by sequencing regions of genomic DNA flanking the ERCC2 gene (Ref. 38). The predicted amino acid sequence of KLC3 suggest that it encodes a kinesin light chain of approximately 56 kDa and is missing the sixth TPR domain. However, it remains to be determined if KLC3 is actually expressed or is a pseudogene. Both mouse KLC2 and mouse KLC3 are possibly in a unique class to themselves and seem equally divergent from kinesin light chains isolated from worms. Although KLCs can be classified into three groups, certain domains of this modular protein are highly conserved. The polypeptide sequences of the known KLCs diverge the most at the C-terminal end and are most highly conserved in the TPR domains. The coiled-coil domain, through which KLC appears to interact with KHC (17), is also conserved although not to the same extent as the TPR domains.

Genetic Mapping of KLC1 and KLC2—The Jackson Laboratory developed backcross panels that utilize polymorphisms between two different mouse species, M. musculus and M. spreitus, to map genes within a centimorgan. Non-cross-hybridizing KLC1 and KLC2 probes were used to find Tag1 polymorphisms using Southern blots of genomic DNA from M. musculus (C57/b16) and M. spreitus. These probes were then used for Southern analysis of 94 offspring from an F1 × M. spreitus backcross.

KLC1 mapped to the distal end of mouse chromosome 12 between Yy1 and D12Bir10 in the Jackson Laboratory chromosomal map. It was predicted to be 4.6 centimorgans distal of Yy1 and 2.2 centimorgans proximal of D12Bir10. This 6.8-centimorgan region was superimposed on a representation of the same region from The Encyclopedia of the Mouse Genome (56) using the nearest common markers in both maps (Yy1 and D12Bir10). KLC1 was in the vicinity of Tnsp2p, Tsu, Thly, Tpre, and Tind on the mouse chromosome. This region is also syntenic with human chromosome 14q32. KLC2 mapped to the proximal end of mouse chromosome 19 between D19Mit32 and Lpc1 in the Jackson Laboratory chromosomal map. KLC2 was predicted to be 1.1 centimorgan distal to D19Mit32 and 13.1 centimorgan proximal to Lpc1. The representative region of localization of KLC2 in the mouse genome map covered 5 centimorgans. This region is syntenic with human chromosome 11q12–11q13 and overlaps with the following mouse genes: Mr66–3, Pth, Pygm, EmK2, Xmnw42, cdc20, adbk1, ly10, Penape2, and Cds5.

A human KLC gene (28) was recloned by another group by reverse transcriptase-PCR of adult human cerebral cortex RNA. This reverse transcriptase-PCR product mapped to human chromosome 14q32.3 by fluorescence in situ hybridization (39), suggesting that KLC1 is the mouse counterpart of this human KLC gene. Sequence analysis of the ERCC2 gene region in human, hamster, and mouse led to the discovery of a kinesin light chain-like sequence in human chromosome 19q13.2–q13.3 (38). This ERCC2 flanking gene is possibly a third KLC gene, since it mapped to mouse chromosome 7.

Expression Pattern of Mouse KLC Transcripts and Proteins—Northern analysis was conducted on tissues from adult mice. Although both KLC1 and KLC2 transcripts were enriched in neuronal tissue, KLC2 was the major kinesin light chain in nonneuronal tissues. Hence, KLC2 was considered to be "ubiquitously" expressed. KLC1 was the minor form of kinesin light chains in all tissues except brain, where its transcript level was equivalent to that of KLC2, and thus it is referred to as "neuronally enriched." Since the Northern blots were done with different probes for KLC1 and KLC2, the intensities of bands in Fig. 2A cannot be directly compared with those in Fig. 2B. However, the relative intensities in different tissues in each blot can be directly compared after quantitation and normalizing for loading (shown in Fig. 2D). Northern blots were also done with embryonic mouse brain from various stages of development (data not shown). Both KLC1 and KLC2 transcripts were expressed at embryonic day 14.5 (earliest time point in the experiment), although the expression level was lower than adult.

Polyclonal antibodies raised against nearly full-length KLC1 and KLC2 were affinity-purified on the cognate antigen and then preadsorbed against the other mouse KLC protein to remove cross-reacting antibodies. Specificity was tested using Western blots of GST fusion protein expressed in Escherichia coli. (Fig. 2, H and I). These non-cross-reacting antibodies were used to probe Western blots of the crude cytoplasmic fraction of various adult mouse tissues. The protein distribution of the two mouse light chains corresponded to their RNA transcript pattern; i.e. KLC1 was predominantly present in tissues of neuronal origin (brain, spinal cord, and sciatic nerve), whereas KLC2 has a relatively ubiquitous pattern of distribution (Fig. 2, compare E and F). It should be noted that the relative levels of KLC1 is more than KLC2 in the crude cytoplasmic fraction of sciatic nerve. However, if the sciatic nerves are dissected and directly solubilized in SDS loading buffer, the levels of KLC1 and KLC2 are comparable. These observations suggest that a large proportion of KLC2 sediments at 3000 × g in sciatic nerve preparations, perhaps because KLC2 either associates with large membranous fractions or forms aggregates. Another possibility might be that tightly wrapped Schwann cells are not as easily homogenized as axons and hence may be selectively sedimented at 3000 × g. Therefore, if KLC2 expression in sciatic nerve is more abundant in Schwann cells compared with axons, centrifugation of the homogenate might lead to selective exclusion of this fraction. The Western experiments were also done with a monoclonal KLC antibody, 63-90 (33), which recognizes both KLC1 and KLC2 (see Fig. 2G). The pattern seen

---

2 J. E. Lamerdin, personal communication.
3 Available on the World Wide Web at http://www.informatics.jax.org/encyclo.html.
with 63-90 reasonably corresponded with that seen with the anti-KLC1 or anti-KLC2 antibodies, except for the band at higher mobility in the case of muscle for KLC2. Thus, the band at higher mobility for KLC2 in muscle is probably spurious. Since 63-90 recognized recombinant KLC1 and KLC2 with equal affinity (data not shown), Western analysis with 63-90 (Fig. 2G) also demonstrates that KLC1 is the major kinesin light chain form in axons of sciatic nerve.

Neither 63-90, anti-KLC1, nor anti-KLC2 recognized a protein of approximately 56 kDa, suggesting that these antibodies do not recognize KLC3, that KLC3 is expressed at very low levels, or that the gene is not transcribed. Attempts to isolate KLC3 sequences by reverse transcriptase-PCR from mouse brain RNA under the same conditions used to isolate KLC2 5'-RACE products proved unsuccessful. These data suggest that if KLC3 is transcribed, the expression level of the protein in brain is significantly lower than that of KLC1 or KLC2.

Interactions of KLC1 and KLC2 with nKHC and uKHC—The identification of two mammalian kinesin light chain genes was interesting in light of the recent finding of two KHC genes in humans (1). This finding raised the possibility that each KLC might only interact with one form of KHC. To address this issue, native kinesin complexes from whole mouse brain lysate were analyzed using immunoprecipitation. Affinity-purified, preadsorbed antibodies against KLC1, KLC2, nKHC, and uKHC were used to immunoprecipitate kinesin complexes from whole brain lysate. The immunoprecipitates from each reaction were then analyzed by subsequent Western blotting with both anti-KHC antibodies and 63-90.

The data (Fig. 3) demonstrate that neither KLC nor KHC form heterodimers in the native complex in brain and that there is no specificity in the interaction of the kinesin light chains with the kinesin heavy chains. When brain lysate is immunoprecipitated with KLC1 antibodies, the kinesin complex contains nKHC, uKHC, and KLC1 but not KLC2. Likewise, the immunoprecipitate from a KLC2 reaction contains nKHC, uKHC, and KLC2 but no KLC1. Immunoprecipitations with nKHC and uKHC bring down both kinesin light chains. The heavy chains, however, do not interact with each other as previously reported (1); nKHC does not coprecipitate with uKHC and vice versa.

Faint bands of uKHC were seen in immunoprecipitates of nKHC; the converse was seen in nKHC immunoprecipitates.

**Fig. 1.** A, comparison of predicted amino acid sequences from KLC1 and KLC2 genes. Genetics Computer Group sequence analysis software (30) was used to predict amino acid sequences of the KLC1 and KLC2 genes, which were then compared using the GAP program. The black box highlights regions believed to form coiled-coils. The gray boxes denote the six TPR regions, of which five are in tandem. The amino acid sequences of the two gene products are 71.1% identical to each other, with most of the identity restricted to the predicted coiled-coil and TPR regions. Sequences diverge at C-terminal ends of the two light chain genes. KLC1 and KLC2 are predicted to encode proteins of approximately 61 and 67 kDa, respectively. B, multiple alignment of KLC sequences. Most protein sequences were from GenBank™. The human KLC2 (38) and mKLC3 sequences corresponded to a possible third form of KLC in mouse from the known chromosomal localization. The mouse KLC3 sequence was predicted from a genomic cosmid fragment and kindly provided by Dr. Jane Lamerdin (38). The two mouse KLC sequences are described in this paper. The protein sequences were aligned, and the dendrogram was created by using the program PILEUP (30). The alternatively spliced forms of rat, squid, sea urchin, and worm KLC sequences were condensed and represented as a single line on the dendrogram (denoted by an asterisk). The two human and three mouse KLC sequences are derived from separate genes. The kinesin light chains are highly conserved between species, with the TPR domains (22) showing the highest degree of conservation. The protein sequences analyzed and GenBank™ accession numbers are as follows: worm wKLC1 (Z29644) and wKLC2 (Z29645) (27); sea urchin sKLC1 (L10233), sKLC2 (L10224), sKLC3 (L10225), and sKLC4 (L08258) (26); squid sKLC362 (L24440), sKLC382, sKLC373, and sKLC352 (L24441) (25); rat rKLCa (M75146), rKLCb (M75147), and rKLCc (M75148) (24); human hKLC (L04733) (28); rat hKLCa (M75146), hKLCb (M75147), and hKLCc (M75148) (24); human hKLC (L04733) (28) and hKLC2 (38); Drosophila dKLC (L11013) (17); mouse mKLC1 (AF056555) and mKLC2 (AF056666) (described here).
reacting antibodies. The hybridization between tissues with KLC1 and KLC2. The amount of radioactivity hybridized to each band was normalized for loading.

E–I 63-90. The upper band gel. Western analysis was done with anti-nKHC (each of the immunoprecipitation reactions were loaded on a 7.5% SDS kinesin complex from whole brain lysate. Equal volumes of lysate and KLC2, nKHC, or uKHC antibodies were used to immunoprecipitate the analysis of KLC1 and KLC2 transcripts. Approximately 15 µg of total RNA (as measured by A260) was loaded per lane. C shows the ethidium bromide staining of the 28 S band. A and B show Northern blots probed with KLC1 and KLC2, respectively. D shows the comparison of relative hybridization between tissues with KLC1 and KLC2. The amount of radioactivity hybridized to each band was normalized for loading. E–I, polyclonal antibodies generated against GST-KLC1 and GST-KLC2 fusion proteins were affinity purified and presorbed to obtain non-cross-reacting antibodies. H and I are Western blots of 1 µg/lane of recombinant GST-KLC1 and GST-KLC2. H was probed with anti-KLC1 antibody, and I was probed with anti-KLC2 antibody. E, F, and G are Western blots of various tissues (loaded at 100 µg/lane except for sciatic nerve, which was loaded at 30 µg/lane) probed with KLC1, KLC2, and 63-90, respectively. 63-90 is a monoclonal antibody that recognizes both mouse KLC proteins (33). KLC1 and KLC2 migrate at approximately 61 and 67 kDa, respectively, and hence the upper band in G corresponds to KLC2, while the lower band is KLC1.

Similarly small amounts of KLC2 were seen in KLC1 immunoprecipitations and vice versa. These contaminants are probably due to low levels of cross-reacting antibodies that were not completely removed by preadsorption. Although the antibodies were checked for complete removal of cross-reacting epitopes on Western blots with recombinant fusion proteins, immunoprecipitation reactions were done under different conditions with native proteins, it is thus likely that most but not all of the cross-reacting antibodies were removed. Double bands seen for nKHC (Fig. 3A) are likely to result from cross-reactivity with a closely related protein or partial degradation. The general conclusions, however, do not change.

These immunoprecipitation experiments also demonstrate that the bands recognized by 63-90 in whole brain lysate are not splice variants of a single KLC gene but rather the gene products of both KLC1 and KLC2.

Localization of KLC1 and KLC2—Cellular localization of KLC1 and KLC2 were analyzed by immunofluorescence experiments with cells or tissues of neuronal origin. HC2S2 cells are hippocampal precursor cells that have been stably transfected with tetracycline-inducible myc (34). In the absence of tetracycline, these cells express Myc and remain in an undifferentiated state. Upon the addition of tetracycline, the cells discontinue Myc production and differentiate into cells with neuronal characteristics. Double label immunofluorescence experiments were done with anti-KLC1 (Fig. 4, A–F) or anti-KLC2 and SMI31 antibodies (Fig. 4, G–L), which recognize phosphorylated NF proteins enriched in axons (40, 41). The data suggest that undifferentiated HC2S2 cells contain less KLC1 than differentiated cells (Fig. 4, compare A with B and C). The levels of KLC2 seem to remain constant between the undifferentiated and differentiated HC2S2 cells (Fig. 4, compare G with H and I). These data were verified by Western analysis of KLC1 and KLC2 content in whole extracts of differentiated and undifferentiated HC2S2 cells (Fig. 4M). HC2S2 cells can differentiate into two major types of neurons that can be distinguished morphologically. One type resembles oligodendrocytes and shows no SMI31 staining, suggesting that these cells do not contain axons (see Fig. 4, E and K). The other type of differentiated HC2S2 cells produce axons, as demonstrated by the presence of phosphorylated NF proteins (see Fig. 4, F and L). The subcellular distribution of KLC1 and KLC2 appears similar in both types of these differentiated cells (Fig. 4, compare B and C with H and I, respectively). SMI31 also stains the nuclei in undifferentiated HC2S2 cells (Fig. 4, D and J); however, this result is probably spurious and idiosyncratic to this cell line.

Since cultured cells are not bona fide representations of in vivo localization, the distributions of KLC1 and KLC2 were also examined in mouse brain tissue. In situ hybridization data

**Fig. 2. Expression pattern of the two mouse light chain genes.** A–D, non-cross-hybridizing radiolabeled probes were used for Northern analysis of KLC1 and KLC2 transcripts. Approximately 15 µg of total RNA (as measured by A260) was loaded per lane. C shows the ethidium bromide staining of the 28 S band. A and B show Northern blots probed with KLC1 and KLC2, respectively. D shows the comparison of relative hybridization between tissues with KLC1 and KLC2. The amount of radioactivity hybridized to each band was normalized for loading. E–I, polyclonal antibodies generated against GST-KLC1 and GST-KLC2 fusion proteins were affinity purified and presorbed to obtain non-cross-reacting antibodies. H and I are Western blots of 1 µg/lane of recombinant GST-KLC1 and GST-KLC2. H was probed with anti-KLC1 antibody, and I was probed with anti-KLC2 antibody. E, F, and G are Western blots of various tissues (loaded at 100 µg/lane except for sciatic nerve, which was loaded at 30 µg/lane) probed with KLC1, KLC2, and 63-90, respectively. 63-90 is a monoclonal antibody that recognizes both mouse KLC proteins (33). KLC1 and KLC2 migrate at approximately 61 and 67 kDa, respectively, and hence the upper band in G corresponds to KLC2, while the lower band is KLC1.

**Fig. 3. Immunoprecipitation analysis of native kinesin.** KLC1, KLC2, nKHC, or uKHC antibodies were used to immunoprecipitate the kinesin complex from whole brain lysate. Equal volumes of lysate and each of the immunoprecipitation reactions were loaded on a 7.5% SDS gel. Western analysis was done with anti-nKHC (A), anti-uKHC (B), or 63-90 (C). The upper band in C corresponds to KLC2, whereas the lower band is KLC1. Immunoprecipitates with anti-uKHC antibody are from a duplicate experiment.

Since cultured cells are not bona fide representations of in vivo localization, the distributions of KLC1 and KLC2 were also examined in mouse brain tissue. In situ hybridization data
suggested that KLC1 and KLC2 transcripts were both enriched in the olfactory bulb, hippocampus, dentate gyrus and in the granular layer of the cerebellum (data not shown). KLC1 showed uniform immunofluorescence staining throughout the brain. Although KLC2 staining was also found throughout the brain, it was highly enriched in the glomeruli of the olfactory bulbs (see Fig. 5, A and C), where the mitral cells and the olfactory nerves synapse. KLC2 staining was also seen in the cell bodies and axons of the mitral cells (see Fig. 6E). A monoclonal antibody 63-90 (33) that recognizes both KLC1 and KLC2 also stained the glomeruli significantly more than the surrounding cells (see Fig. 5G), whereas the anti KLC1 antibody did not specifically stain these structures (see Fig. 5H).

The peripheral nervous system was examined, since it provides a system with axons spatially isolated from the cell bodies. Double labeling experiments with 63-90 and KLC1 (Fig. 6A) clearly demonstrated that KLC1 staining is diffuse and limited to axons within the sciatic nerve. 63-90, which sees both KLC1 and KLC2, stained axons and Schwann cells of the sciatic nerve, suggesting that KLC2 is the major kinesin light chain present in Schwann cells (Fig. 6, B and D). Staining within the axons with 63-90 looked more diffuse than punctate (Fig. 6, B and D). Specific KLC2 staining, however, also localizes within the axons in a punctate pattern (see Fig. 6C, inset) and is present in high levels in the Schwann cells encompassing the axons (Fig. 6C). Double labeling experiments were also performed with KLC1 and either SMI31 or S100 (Sigma; antibody marker against Schwann cells) to demonstrate that KLC1 is expressed in the axons but not the Schwann cells of the sciatic nerve (see Fig. 7). KLC1 is present in a subset of cells in the sciatic nerve and the staining seems to correspond with phosphorylated NF (Fig. 7, compare C and D) but not Schwann cells (Fig. 7, compare A and B). Since Schwann cells tightly wrap around the axons, the presence of KLC1 only in the axons of the sciatic nerve was confirmed in cross-sections through the sciatic nerve (Fig. 7, compare E and F with G and H).

Biochemical Fractionation of Whole Brain Extract—Biochemical fractionation studies, combined with immunofluorescence data, can give clues to the subcellular distribution of a protein. Cell fractionation studies were carried out as described (35). Briefly, fractions corresponding to cytosol, microsomes, crude synaptosomes, and mitochondria were prepared and subjected to Western blotting. The 63-90 monoclonal antibody was used to compare the distribution of the different light chain products within the various fractions. The majority of KLC1 and KLC2 is seen in the S3 cytosolic fraction (Fig. 8A), suggesting that a large proportion of these proteins are soluble. This pattern corresponded to that seen with the monoclonal
KLC1 and KLC2—Microtubule Binding Properties of Kinesin Containing KLC1 and KLC2—Taxol stimulated microtubule assembly and sedimentation have been used to purify conventional kinesin from a variety of sources. Since native kinesin is a complex of kinesin heavy chains and kinesin light chains, kinesin light chains cosediment with stabilized microtubules. In the presence of AMP-PNP, a nonhydrolyzable analog of ATP, both KLC1 and KLC2 cosediment with taxol-stabilized microtubules from mouse brain homogenates (see Fig. 9A). The kinesin light chains are released from microtubules in the presence of either high salt concentrations or ATP (Fig. 9B).

Indeed, the general sedimentation profile of the kinesin heavy chains is identical to that found with KLC1 (data not shown). Although the binding patterns of KLC1 and KLC2 are quite similar, there are significant differences in the sedimentation profiles of KLC1 and KLC2. KLC1 only associates with stabilized microtubules in the presence of AMP-PNP, and this association is easily reversed by high salt concentrations or ATP. The sedimentation profile seen with KLC2 suggests that a subset associates with stabilized microtubules even in the presence of high salt or ATP. However, since a subset of KLC2 also sediments in the absence of stabilized microtubules (Fig. 9A, No GTP, No Taxol lane) these differences are very likely to be microtubule-independent.

**DISCUSSION**

Multiple KLC Genes in Mice—Although several different kinesin heavy chain genes were identified in *M. musculus* (43–45), only one gene for kinesin light chain has been cloned from various nonmammalian sources (25–27). In humans, Niclas et al. (1) reported two kinesin heavy chain genes; one is expressed in a neuronal distribution, while the other was present in an ubiquitous manner. The identification of multiple mouse kinesin light chain genes, the KLC1, KLC2, and KLC3 genes, of which one, the KLC1 gene, is predominantly expressed in neuronal tissue, is especially interesting in light of these findings. Given the sequence similarities of KLC1, KLC2, and KLC3 and the high degree of conservation of the coiled-coil and TPR domains, it is likely that these genes evolved from a duplication of a common ancestral gene. Since *Drosophila* and *C. elegans* appear to have one gene for kinesin light chain, the evolution of a neuronally enriched kinesin light chain gene might be a recent event. Interestingly, native kinesin from *Neurospora crassa* does not contain any copurifying light chains (46). The need for higher organisms to generate kinesin light chains may have resulted from initial development of a relatively simple transport system in invertebrates followed ultimately by a complex nervous system in mammals. Perhaps the complexity of the mammalian nervous system required a sizable increase in amount or character of transport, which in turn necessitated the evolution of neuronally enriched kinesin light chain. The worm kinesin light chains, mouse KLC3, and its human homologue (human KLC2) seem to be an anomaly in this seemingly structured evolution of kinesin light chains. Thus, it will be especially interesting to examine if KLC1 plays a unique role in neuronal tissue.

KLC1 and KLC2 Exhibit Different Properties—In neurons, many cargoes are transported from the cell body to the synapse. Thus, an efficient cellular transport mechanism is required, possibly involving several families of molecular motors, each performing a unique function. At least 10 different kinesin-like molecules have already been identified (37, 43, 45, 47–51) in mouse brain, and more remain to be discovered. Given the complex organization of the mammalian neuron, it is possible that the two kinesin light chains in mouse perform different functions within the cell, although they may be functionally redundant.

Experiments described in this paper support the former scenario. In the peripheral nervous system, KLC1 is found only in axons, whereas KLC2 is found in axons and Schwann cells. Immunofluorescence staining also shows that KLC1 distribution is diffuse, whereas anti-KLC2 antibodies stain punctate structures in the axons. More noticeably, KLC1 but not KLC2 levels are elevated when HC2S2 cells (34) are induced to differentiate into neurons. Cellular fractionation studies also indicate that KLC2 is present at a higher level than KLC1 in microsomal fractions.

Unlike the neuronal heavy chain gene product, KLC1 is not restricted to cells of neuronal origin, suggesting that complex interactions between KLC and KHC exist in mammalian systems. In fact, immunoprecipitation experiments show that although KLC and KHC are likely to form homodimers among themselves, the interactions between these two subunits are not specific. NKHC and uKHC subcellular localization is compartmentalized in neuronal cells; nKHC is found predominantly in the cell bodies, while uKHC is found in the processes.
of the neuron (1). The differences in distribution of KLC1 and KLC2 are not as striking as that seen with nKHC and uKHC in the central nervous system; however, there are substantial differences seen in the peripheral nervous system. Furthermore, in cultured neurons KLC1, but not KLC2, was seen to be up-regulated upon differentiation.

**Functional Diversity of Kinesins**—The biochemical data suggest that there are at least four different forms (nKHC-KLC1, nKHC-KLC2, uKHC-KLC1, and uKHC-KLC2) in the mouse nervous system. The likelihood that the two mouse KLC transcripts undergo alternative splicing to produce numerous other isoforms similar to that seen in other organisms (24–27), suggests that various combinational associations of the kinesin subunits may produce many functionally diverse holoenzymes. The presence of various mammalian kinesin holoenzymes offers the possibility of selectively associating with different cargoes or even modulating the rate of motility of the various forms. In fact, it has been shown that kinesin travels in two peaks in fast axonal transport (52). Furthermore, biochemical and immunofluorescence studies show that kinesin colocalizes with a wide variety of intracellular structures and possible cargoes (reviewed in Refs. 53 and 54). A likely explanation for these observations is that each of the four kinesin holoenzymes performs slightly different functions. However, the possibility of functional redundancy also arises, ranging from slight overlap lapping to completely redundant roles. Genetic experiments using homologous recombination (55) can be used to test these ideas.

**Acknowledgments**—We thank Drs. David Stenoien and Scott Brady (University of Texas, Southwestern Medical Center) for sharing monoclonal anti-rat KLC antibodies including 63-90, prior to publication; Dr. Ron Vale (University of California, San Francisco) for a constant supply of affinity-purified/preadsorbed uKHC and nKHC antibodies, and Dr. Rusty Gage (Salk Institute) for HC2S2 cells. We thank Dr. Jane Lamerdin (Lawrence Livermore National Laboratory) for providing the sequence for mouse KLC3. Bruce Ritchings assisted with sequencing the 5’-RACE product of the KLC2 gene and Drs. Maura McGrail and Heiner Mattheis helped tremendously with confocal microscopy.

**REFERENCES**

1. Niclas, J., Navone, F., Hum-Bohor, N., and Vale, R. D. (1994) Neuron 12, 1059–1072
2. Vale, R. D., Reese, T. S., and Sheetz, M. P. (1985) Cell 42, 39–50
3. Brady, S. T. (1985) Nature 317, 73–75
4. Pfister, K. K., Wagner, M. C., Stenoien, D. L., Brady, S. T., and Bloom, G. S. (1989) J. Cell Biol. 108, 1453–1463
5. Brady, S. T., Pfister, R. K., and Bloom, G. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1061–1065
6. Hollenbeck, P. J., and Swanson, J. A. (1996) Nature 346, 864–866
7. Hirokawa, N., Sato-Yoshitake, R., Kobayashi, N., Pfister, K. K., Bloom, G. S.,
and Brady, S. T. (1991) J. Cell Biol. 114, 295–302
8. Rodionov, V. I., Gyoerffy, F. K., and Gelfand, V. I. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4956–4960
9. Gho, M., McDonald, K., Ganetzky, B., and Saxton, W. M. (1992) Science 258, 313–316
10. Hall, D. H., Plenefisch, J., and Hedgecock, E. M. (1991) J. Cell Biol. 115, 389 (abstr.)
11. Ferreira, A., Nielas, J., Vale, R. D., Banker, G., and Kosik, K. S. (1992) J. Cell Biol. 117, 585–606
12. Amaratunga, A., Mertin, P. J., Kosik, K. S., and Fine, R. E. (1993) J. Biol. Chem. 268, 17427–17430
13. Bloom, G. S., Wagner, M. C., Pfister, K. K., and Brady, S. T. (1988) Cell 56, 879–889
14. de Cuevas, M., Tao, T., and Goldstein, L. S. B. (1992) J. Cell Biol. 116, 957–965
15. Yang, J. T., Laymon, R. A., and Goldstein, L. S. B. (1993) J. Cell Biol. 126, 13657–13666
16. Hirokawa, N., Pfister, K. K., Yorifuji, H., Wagner, M. C., Brady, S. T., and Bloom, G. S. (1989) Cell 56, 875–878
17. Gauger, A. K., and Goldstein, L. S. B. (1993) J. Biol. Chem. 268, 13657–13666
18. Hirokawa, N., Pfister, K. K., Yorifuji, H., Wagner, M. C., Brady, S. T., and Bloom, G. S. (1989) Cell 56, 875–878
19. Skoufias, D. A., Cole, D. G., Wedaman, K. P., and Scholey, J. M. (1994) J. Biol. Chem. 269, 1477–1485
20. Bi, G. Q., Moris, R. L., Liao, G., A. M., Scholey, J. M., and Steinhardt, R. A. (1996) J. Cell Biol. 138, 999–1008
21. Yang, J. T., Saxton, W. M., Stewart, R. J., Raff, E. C., and Goldstein, L. S. B. (1990) Science 249, 42–47
22. Giedroc, J. G. J., and Goldstein, L. S. B. (1996) Trends Biochem. Sci. 21, 52–53
23. Lamb, J. R., Treudreich, S., and Hieter, P. (1995) Trends Biochem. Sci. 20, 257–265
24. Cyr, J. L., Pfister, K. K., Bloom, G. S., Slaughter, C. A., and Brady, S. T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10114–10118
25. Beushausen, S., Kladas, A., and Jaffe, H. (1993) DNA Cell Biol. 12, 901–909
26. Wedaman, K. P., Knight, A. E., Kendrick, J. J., and Scholey, J. M. (1993) J. Mol. Biol. 231, 155–158
27. Fan, J., and Amos, L. A. (1994) J. Mol. Biol. 240, 567–512
28. Okazaki-Arvela, Y., Shih, L. C., Hardman, N., Asselbergs, F., Bille, G., Schmitz, A., White, B., Siciliano, M. J., and Lachman, L. B. (1993) DNA Cell Biol. 12, 881–892
29. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
30. Devereux, J., Haeberli, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395
31. Guan, K. L., and Dixon, J. E. (1991) Anal. Biochem. 192, 262–267
32. Laemmli, U. K. (1970) Nature 227, 680–685
33. Stenson, D. L., and Brady, S. T. (1997) Mol. Biol. Cell 8, 675–689
34. Hoshimaru, M., Ray, J., Sah, D. W., and Gage, F. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1518–1523
35. Arai, M., and Cohen, J. A. (1994) J. Neurosci. Res. 38, 345–357
36. Barton, N. R., Pereira, A. J., and Goldstein, L. S. B. (1995) Mol. Biol. Cell 6, 1563–1574
37. Hanlon, D. W., Yang, Z., and Goldstein, L. S. B. (1997) Neuron 18, 439–451
38. Lamerin, J. E., Stillwagen, S. A., Ramirez, M. H., Stubbs, L., and Carrano, A. V. (1996) Genomics 34, 399–409
39. Seidert, M., Marsh, S., and Carter, N. (1996) Genomics 32, 173–175
40. Sterrenberger, L. A., and Sterrenberger, N. H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6126–6130
41. Matus, A. (1998) Trends Neurosci. 21, 291–292
42. Ingold, A. L., Cohn, S. A., and Scholey, J. M. (1998) J. Cell Biol. 107, 2657–2667
43. Aizawa, H., Sekine, Y., Takemura, R., Zhang, Z., Nagakura, M., and Hirokawa, N. (1992) J. Cell Biol. 119, 1287–1296
44. Guzik, A. V., Kazarov, A. R., Thimmappaya, R., Axenovich, S. A., Mazo, I. A., and Roninson, I. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3744–3748
45. Kato, K. (1991) Eur. J. Neurosci. 2, 704–711
46. Steinberg, G., and Schliwa, M. (1995) Mol. Biol. Cell 6, 1605–1618
47. Nangaku, M., Sato-Yoshitake, R., Okada, Y., Noda, Y., Takemura, R., Yamazaki, H., and Hirokawa, N. (1994) Cell 79, 1209–1220
48. Kondo, S., Sato-Yoshitake, R., Noda, Y., Aizawa, H., Nakata, T., Matsuura, Y., and Hirokawa, N. (1994) J. Cell Biol. 125, 1095–1107
49. Yamazaki, H., Nakata, T., Okada, Y., and Hirokawa, N. (1995) J. Cell Biol. 130, 1387–1399
50. Yang, Z., and Goldstein, L. S. B. (1998) Mol. Biol. Cell 9, 249–261
51. Okada, Y., Yamazaki, H., Sekine-Aizawa, Y., and Hirokawa, N. (1995) Cell 81, 769–780
52. Elluru, R. G., Bloom, G. S. and Brady, S. T. (1995) Mol. Biol. Cell 6, 21–40
53. Goldstein, L. S. B. (1993) Annu. Rev. Genet. 27, 319–351
54. Bloom, G. S., and Endow, S. A. (1994) Protein Profile 1, 1059–1116
55. Caperi, M. R. (1989) Science 244, 1288–1292
56. The Jackson Laboratory, The Encyclopedia of the Mouse Genome, Release 3.0, The Jackson Laboratory, Bar Harbor, ME
Two Kinesin Light Chain Genes in Mice: IDENTIFICATION AND CHARACTERIZATION OF THE ENCODED PROTEINS
Amena Rahman, Dara S. Friedman and Lawrence S. B. Goldstein

J. Biol. Chem. 1998, 273:15395-15403.
doi: 10.1074/jbc.273.25.15395

Access the most updated version of this article at http://www.jbc.org/content/273/25/15395

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 54 references, 26 of which can be accessed free at http://www.jbc.org/content/273/25/15395.full.html#ref-list-1
Additions and Corrections

Vol. 273 (1998) 19817–19821

Cooperation of a single lysine mutation and a C-terminal domain in the cytoplasmic sequestration of the p53 protein.

Shun-Hsin Liang, David Hong, and Michael F. Clarke

Pages 19819, Fig. 1: The next to the last sentence in the figure legend is incorrect. The correct version is shown below:

MCF-7 cells were transfected with the various mutated p53 DNA and GFP fusions including p53Δ1-300 (c), p53Δ1-305 (d), p53K305N (e), p53K305NΔ351-393 (f), p53K305NΔ356-393 (g), and p53K305NΔ326-335 (h).

Vol. 273 (1998) 15395–15403

Two kinesin light chain genes in mice. Identification and characterization of the encoded proteins.

Amena Rahman, Dara S. Friedman, and Lawrence S. B. Goldstein

Page 15398, legend to Fig. 1: The accession number for mouse mKLC1 is incorrect. The correct number is AF055665.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.