Suppression of Kinesin Expression in Cultured Hippocampal Neurons Using Antisense Oligonucleotides

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Abstract. Kinesin, a microtubule-based force-generating molecule, is thought to translocate or ganelles along microtubules. To examine the function of kinesin in neurons, we sought to suppress kinesin heavy chain (KHC) expression in cultured hippocampal neurons using antisense oligonucleotides and study the phenotype of these KHC “null” cells. Two different antisense oligonucleotides complementary to the KHC sequence reduced the protein levels of the heavy chain by greater than 95% within 24 h after application and produced identical phenotypes. After inhibition of KHC expression for 24 or 48 h, neurons extended an array of neurites often with one neurite longer than the others; however, the length of all these neurites was significantly reduced. Inhibition of KHC expression also altered the distribution of GAP-43 and synapsin I, two proteins thought to be transported in association with membranous organelles. These proteins, which are normally localized at the tips of growing neurites, were confined to the cell body in antisense-treated cells. Treatment of the cells with the corresponding sense oligonucleotides affected neither the distribution of GAP-43 and synapsin I, nor the length of neurites. A full recovery of neurite length occurred after removal of the antisense oligonucleotides from the medium. These data indicate that KHC plays a role in the anterograde translocation of vesicles containing GAP-43 and synapsin I. A deficiency in vesicle delivery may also explain the inhibition of neurite outgrowth. Despite the inhibition of KHC and the failure of GAP-43 and synapsin I to move out of the cell body, hippocampal neurons can extend processes and acquire an asymmetric morphology.

Kinesin, a microtubule-based motor protein, is a heterotetramer consisting of two 110–130 kD heavy chains and two 60–70 kD light chains (Bloom et al., 1988; Kuznetsov et al., 1988). The primary structure of the heavy chain is conserved in four widely divergent species: Drosophila (Yang et al., 1989), squid (Kosik et al., 1990), sea urchin (Wright et al., 1991), and human (Navone et al., in press). Purified kinesin generates ATP-dependent microtubule gliding after being adsorbed onto glass coverslips (Vale et al., 1985a; Scholey et al., 1985; Porter et al., 1987) or induces the transport of latex beads along microtubules after adsorption onto the bead surface (Vale et al., 1985a). In experiments with purified bovine chromaffin granules, the addition of kinesin and ATP was sufficient to induce translocation of the granules along microtubules (Uruttia et al., 1991). Kinesin-coated objects move unidirectionally towards the plus end of microtubules (Vale et al., 1985b; Porter et al., 1987). This direction corresponds to anterograde transport in neurons, since axonal microtubules have their plus ends oriented distally (Filliatreau and DiGiamberdino, 1981; Heidemann et al., 1981; Baas et al., 1988).

Several experiments also support the idea that kinesin transports organelles in vivo. Microinjection of antibodies to kinesin heavy chain (KHC) inhibit tubular lysosome extension in macrophages (Hollenbeck and Swanson, 1990), pigment granule dispersion in melanophores (Rodionov et al., 1991) and organelle transport in extruded axoplasm (Brady et al., 1990). An association of kinesin with membrane-bound organelles was suggested by the immunofluorescence localization of KHC to punctate and tubular Triton X-100 soluble structures (Pfister et al., 1989; Hollenbeck, 1989; Brady et al., 1990; Wright et al., 1991; Houliston and Elison, 1991). Higher resolution analysis by immunogold EM has shown that KHC is associated with membrane-bound organelles (Hirokawa et al., 1991). Furthermore, after ligation of a peripheral nerve, organelles that react with KHC antibodies collect on the proximal side of the ligation, implying that kinesin is associated with anterogradely moving organelles (Hirokawa et al., 1991).

Some clues concerning the in vivo functions of kinesin

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1. Abbreviations used in this paper: KHC, kinesin heavy chain.

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were obtained by examining the phenotype of KHC mutants. A *Drosophila* containing temperature-sensitive KHC mutant had impaired muscle activity and tactile sensitivity, most pronounced in the posterior region (Saxton et al., 1991). Mutations of the *unc-104* locus in *C. elegans*, which encodes a kinesin-like molecule (Otsuka et al., 1991), also cause deficits in neuromuscular transmission, which appear to be secondary to a deficit in anterograde transport. These mutants exhibit a severe depletion of synaptic vesicles in presynaptic terminals and a corresponding accumulation of vesicles in nerve cell bodies (Hall and Hedgecock, 1991). Despite this defect, neurites are able to grow out and establish appropriate synaptic connections.

In this study, we examined the cellular functions of kinesin in mammalian neurons. To approach this problem, we created a pseudo-genetic null mutation of KHC in cultured rat hippocampal neurons by administering antisense oligonucleotides to suppress KHC expression. Cells treated with the KHC antisense oligonucleotides extended neurites which elongated and expressed polarity, but the neurites were significantly shorter than in untreated cells. In addition, neurons treated with KHC antisense oligonucleotides did not transport GAP-43 or synapsin I to the neurite tip, suggesting that kinesin is required for the normal anterograde transport of these molecules.

**Materials and Methods**

**Cultures**

Neuronal cultures were prepared from the hippocampi of embryonic day 18 rats as previously described (Bartlett and Banker, 1984; Goslins and Banker, 1991). In brief, cells from the dissected hippocampi were dissociated by trypsinization (0.25% for 15 min at 37°C) followed by trituration with a fire polished Pasteur pipette. For immunofluorescence, the cells were plated at a density of 100,000 cells per 60-mm petri dish on glass coverslips coated with poly-L-lysine (1 mg/ml) in MEM with 10% horse serum. In antisense experiments, cells were plated onto 12-mm diameter coverslips that were placed with the cells facing up in 35-mm dishes with 1 ml of medium. After 2-4 h the medium was changed to MEM with N2 supplements (Bottenstein and Sato, 1979), ovalbumin (0.1%), and pyruvate (0.01 mg/ml) that had been conditioned in cultures of astroglial cells for 24 h. Long-term cultures used in immunolocalization studies were co-cultured with astroglia.

**Antisense Oligonucleotides**

The initial experiments were performed with oligonucleotides (−11+14 hkin) based upon the sequence of the human KHC (Navone et al., In press). Because the neuronal cultures were taken from rat brain, these experiments were subsequently repeated using oligonucleotides corresponding to the rat KHC sequence. The rat sequence in the region of the initiator codon AUG was obtained by using nondegenerate, 18-mer human primers and performing polymerase chain reaction (PCR) on reverse transcribed rat brain mRNA. An amplified band of the expected size was obtained and the DNA was cloned and sequenced. Key experiments were repeated with rat sense and antisense oligonucleotides.

Antisense oligonucleotide −11+14 hkin, consisting of the sequence GCCAGGTGCCCATCTTCTTCCGAC, is the inverse complement of the human nucleotides −11 to +14. Antisense oligonucleotide −11+14 rkin, consisting of the sequence GCCGGTGTTGCCATCTTCTTCCGAC, is the inverse complement of the rat nucleotides −11 to +14. Antisense oligonucleotide −35−12 rkin, consisting of the sequence CCGGGACTG-

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Results

Immunolocalization of KHC in Cultured Rat Hippocampal Cells

Cultured rat hippocampal neurons from E18 rat brain represent a relatively homogeneous population of neurons in which pyramidal cells predominate. After plating, hippocampal neurons undergo predictable developmental changes (Dotti et al., 1988). At stage I, during the first few hours after plating, the neurons appear rounded. Stage II (Fig. 1) begins ~8 h after plating, when the neurons elaborate a symmetric array of neurites. After ~24 h in culture, one of the processes rapidly elongates (stage III); this process is the axon. During stage IV (72–96 h in culture), the remaining neurites differentiate into dendrites. Kinesin reactivity was observed at all of these stages (Fig. 1). It appeared to be homogeneously distributed, as was tubulin. Kinesin immunoreactivity was present in cell bodies and in both axonal and dendritic processes.

Antisense Oligonucleotide Affect KHC Expression

The oligonucleotides (antisense and sense) were tested for their ability to inhibit KHC expression. The sequence sites selected for the design of the oligonucleotides were centered on the initiator AUG and a nonoverlapping site located immediately upstream in the 5' untranslated region. Cells incubated with each of the antisense oligonucleotides described in Materials and Methods showed markedly reduced reactivity to a KHC mAb (SU4K), as assessed by immunofluorescence at 24 or 48 h after plating (Fig. 2). Neurons incubated with the sense oligonucleotides, on the other hand, appeared unaffected and showed a normal degree of KHC immunoreactivity by immunofluorescence.

To verify that KHC was depleted from cells after antisense treatment, the more sensitive technique of dot immunobinding was used for detection (Fig. 3). KHC was not detectable above the background level of counts in cells grown in the presence of the antisense oligonucleotide -11+14 hkin by the dot immunobinding assay. In contrast, cells treated with sense oligonucleotides were comparable in their immunoreactivity to untreated control cells. Exposure to the antisense oligonucleotides did not affect tubulin and dynein immunoreactivity by the same assay (Fig. 3). The presence of normal levels of tubulin and dynein in the kinesin-suppressed cells suggests that the effect of the antisense oligonucleotide was specific and that the regulation of the expression of the retrograde motor, dynein, was independent of kinesin.

Effects of Antisense Oligonucleotides on Neurite Extension

Neurite extension was measured after either a 24- or 48-h exposure to the KHC oligonucleotides. Neurite elongation during the second 24-h period is considerably more affected by the antisense oligonucleotide treatment than during the first 24-h period (Fig. 4). At both time points nearly all of the neurons had two populations of neurites consisting of several short minor neurites and a single long neurite considered the axon. When the cells were exposed to the antisense oligonucleotide -11+14 hkin and fixed 24 h after plating, there was a 25% decrease in the lengths of the minor neurites and a 34% decrease in the length of the presumptive axon. The mean length of the presumptive axon was 74.5 ± 2.1 µm (compared with 111.7 ± 5.6 µm in sense-treated controls) and the summed length of the minor neurites was 79.6 ± 4.1 µm (compared with 105.2 ± 5.5 µm in sense-treated controls). Neurite growth of neurons exposed to KHC antisense oligonucleotides for 48 h is quantitated in Table I. Neurons treated with kinesin antisense oligonucleotides (50 µM) for 48 h exhibited a 50% decrease in both the lengths of the minor processes and the axon-like processes. The human antisense oligonucleotide was slightly less effective than the rat antisense oligonucleotide, but the difference was not statistically significant. Therefore, the imperfect match of the human antisense oligonucleotide, which differed in two nucleotides, did not significantly affect KHC suppression. On average the antisense-treated neurons had one less minor neurite than the sense-treated controls (Table I).

The KHC antisense oligonucleotides affected neurite outgrowth in a dose-dependent manner (Fig. 5). Increasing the concentration of the antisense oligonucleotide from 12.5 to 50 µM caused a progressive decrease in neurite outgrowth. At 12.5 µM the total neurite length was reduced 19% and at 50 µM it was reduced 50%.

The KHC antisense oligonucleotides, while suppressing KHC expression, did not irreversibly damage the neurons (Fig. 6). When, after 48 h, the cells were released from antisense inhibition by changing the medium, neurite extension resumed at a rate that paralleled that observed under control conditions.

Distribution of GAP-43 and Synapsin in Antisense-treated Neurons

Since kinesin has been postulated to power anterograde axonal transport, we examined the effect of KHC antisense oligonucleotides on the immunocytochemical distribution of GAP-43 and synapsin, molecules that are transported to nerve termini by fast axonal transport. Under control conditions, GAP-43 was present at the tips of neurites and within the cell bodies of neurons after 48 h in culture (Fig. 7). Very little staining was detectable along the shafts of neurites. This pattern was similar to that described in Fig. 2 H of Goslin and Banker (1990). Likewise, synapsin I, under control conditions, was concentrated in the distal portion of the axon (Fig. 8), as described previously in hippocampal neurons (Fletcher et al., 1991). Following exposure to KHC antisense oligonucleotides, the GAP-43 and synapsin I immunoreactivities were largely restricted to the cell body; the neurites were essentially devoid of immunostaining (Fig. 7 and 8).

Discussion

Phenotype of Hippocampal Neurons After Kinesin Suppression

A powerful approach for understanding a protein's cellular function is to study the phenotype of a cell or organism deficient in that protein. Targeted gene deletion is the most widely used strategy for achieving these goals; however, such experiments are not readily possible in postmitotic neurons. Messenger RNA inactivation, either by expression of antisense RNAs or exposure to antisense oligonucleotides,
Figure 2. Hippocampal neurons fixed after 48 h in culture under sense (A and B) and antisense (C and D) conditions. The cells were double labeled with antibodies to tubulin (A and C) and kinesin SUK4 (B and D). The oligonucleotides used in this experiment were hkin -11+14 (sense) and -11+14 hkin (antisense). Identical results were observed with the other oligonucleotides. ax, axon; mp, minor process. Bar, 20 μm.
provides an alternative means of reducing cellular concentrations of a specific protein. In this study, we have shown that exposure to antisense oligonucleotides causes a marked reduction in the levels of the KHC protein in rat hippocampal neurons. After a 24-h exposure to antisense, but not to sense oligonucleotides, the levels of KHC protein are not detectable above background by dot-immunobinding. Thus, antisense oligonucleotide treatment provides a means of creating a "null" kinesin phenotype in a mammalian neuronal cell.

The homogeneous pattern of KHC labeling in the cultured neurons is consistent with the reported large soluble pool (Hollenbeck, 1989). However other investigators have observed a punctate pattern consistent with localization to membranous organelles (Pfister et al., 1989; Wright et al., 1991). It seems likely that the punctate pattern results from some degree of extraction of KHC with the fixatives used. Brady (1991) has raised a number of plausible arguments that favor a predominant membrane localization for KHC, and has reconciled the solubility of the molecule by proposing an easily extractable relationship to membranes. The issue, however, remains problematic because recent data using ex-truded squid axoplasm suggests a tight association of plus-end motors with organelles that does not exchange with the soluble pool (Schnapp, B. J., T. S. Reese, and R. B. Bechtold. 1991. J. Cell. Biol. 115:40 Abstr.)

Several results indicate that the phenotypic changes in hippocampal cells observed after KHC antisense oligonucleotide treatment can most likely be attributed to the reduced levels of KHC. First, the antisense oligonucleotides do not cause irreversible damage to the cell; neurons resume neurite outgrowth at normal rates following a change to medium free of the antisense oligonucleotide. Second, two different antisense oligonucleotides complementary to two different regions of the KHC mRNA produce identical morphological effects on hippocampal neurons. The finding reduces the likelihood that the observed cellular effects are because of the oligonucleotides hybridizing to an mRNA other than the KHC mRNA. Among the kinesin-like molecules sequenced to date, the most significant region of homology lies in the
ATP- and microtubule-binding sites, not the region at the 5' end from where we derived our antisense oligonucleotides. Third, tubulin and dynein protein levels remain normal suggesting that there is not a general reduction in protein synthetic activity.

The phenotype of KHC suppressed hippocampal neurons offers some insight into the normal physiological roles for kinesin in these cells. One striking finding is the altered distribution of synapsin I and GAP-43 in cells treated with KHC antisense oligonucleotides. In untreated cells or cells treated with sense oligonucleotides, synapsin I and GAP-43 are found in the neurites and are particularly concentrated in the termini. In antisense-treated cells, on the other hand, both proteins are confined to the cell bodies. Synapsin I and GAP-43 are both membrane-associated proteins (De Camilli et al., 1983; Valtorta et al., 1988; Gispen et al., 1985) and are

Figure 6. Recovery from antisense oligonucleotide treatment. The neurons in A were treated with kinesin sense oligonucleotides and in B with kinesin antisense oligonucleotides for 48 h. Then medium was changed and oligonucleotides were excluded. At 72 h the cells were fixed and stained with kinesin antibody (SUK4). Although the growth of the cell in B appears to lag behind the cells in A, recovery of kinesin immunoreactivity and morphological development is evident. Bar, 20 μm.
delivered to neuronal termini by fast axonal transport (Baitinger and Willard, 1987; Skene and Willard, 1981; Benowitz et al., 1981). Microtubules in both minor neurites and axons are aligned with their plus ends distal to the cell body (Baas et al., 1989), so that translocation of organelles to the neurite terminus of both types of processes requires a plus-end-directed motor. The impaired delivery of synap-
sin I and GAP-43 to the neurite terminus under antisense conditions suggests that kinesin may serve as the plus-end motor involved in the anterograde transport of vesicles containing these proteins. This conclusion is consistent with recent findings that KHC is associated with vesicles traveling by anterograde transport that accumulate at the proximal side of a nerve ligation (Hirokawa et al., 1991). Alterna-

Figure 7. GAP-43 staining. A and B are kinesin sense oligonucleotide treated. C and D are kinesin antisense oligonucleotide treated. A and C are stained with the tubulin antibody and B and D are stained with the GAP-43 antibody (9-IE12). Bar, 20 μm.
Figure 8. Synapsin I staining. A and B are kinesin sense oligonucleotide treated. C and D are kinesin antisense oligonucleotide treated. A and C are stained with the tubulin antibody (DMIB) and B and D are stained with the synapsin I rabbit antibody. Bar, 20 μm.
The specific suppression of KHC expression by antisense technology, it is formally possible that KHC is unnecessary for the transport of GAP-43 and synapsin I, but instead inhibits the transport of another protein that traps GAP-43 and synapsin I in distal neurites.

Another consistent change in the antisense-treated cells was a reduction in neurite length that affected both the axon and the minor processes which will become dendrites. Both classes of neurites were reduced in length by \( \sim 50\% \) after 48 h in antisense-containing medium, but resumed a normal growth rate upon removal of the oligonucleotides. The greater effect on the minor neurites after 48 h compared to just 24 h in the antisense-containing media suggested that the longer the processes grew, the more dependent they became on kinesin for further elongation (Fig. 4). It is generally thought that the addition of new surface membrane necessary for neurite elongation occurs predominantly at growth cones (Pfenninger and Maylie-Pfenninger, 1981). Thus impaired anterograde transport of vesicles in KHC null cells may be the underlying cause for the reduced rate of process outgrowth.

If this explanation is correct, why is neurite elongation not entirely blocked? It may be that neurite outgrowth can occur in the absence of anterograde vesicle transport to growth cones. Membrane addition within the cell body could be sufficient to support a reduced rate of process outgrowth, at least for short distances (Small et al., 1984) and in fish epidermal keratocytes there is a rapid forward transport of concanavalin A-coated gold particles on the dorsal surfaces of lamellipodia that is blocked by treatment with cytochalasin D (Kucik et al., 1989). An alternative possibility is that a different anterograde motor drives the transport of other classes of organelles that can, in part, support neurite elongation. In Drosophila, for example a dozen or more different kinesin homologs have been identified (Endow and Hatsumi, 1991).

The particular class of organelles translocated by specific motors remains problematic, particularly in comparing diverse systems. Some degree of organelle specificity was suggested for the putative motor gene unc-104 in C. elegans because neurons from the mutant phenotype accumulated synaptic vesicles in nerve cell bodies and were depleted of such vesicles in presynaptic terminals (Hall and Hedgecock, 1991). The protein encoded by the unc-104 gene bears homology to KHC only in the force-generating head domain (Otsuka et al., 1991). Unc-116 is a distinct locus, which encodes a kinesin that is 55% identical over its entire length to the Drosophila and squid KHC (de Feo, G., N. Patel, and J. R. Mancillas. 1991. Soc. Neurosci. Abstr. 17:58). One allele at this locus has defects restricted to the nervous system (de Feo, G., N. Patel, and J. R. Mancillas. 1991. Soc. Neurosci. Abstr. 17:58), and this mutant may represent a closer analogy to the defect in the oligonucleotide-induced KHC null neurons.

The ultimate loss of ribosomes during the transformation of a minor neurite into an axon, and thus the loss of a basis for local protein synthesis, places a high priority on axonal transport to support this rapidly elongating structure. However, some degree of process outgrowth might be a consequence primarily of the polymerization and/or sliding of microtubules. Microtubule polymerization can produce work in vitro (Hotani and Miyamoto, 1990) and appears to drive process formation in SF9 cells that express high levels of \( \tau \) protein (Knopps et al., 1991). Furthermore, recent studies (Tanaka and Kirschner, 1991) indicate that intact microtubules are pushed anterograde in neurons; such sliding forces may also contribute to process extension. It will be important to examine the growing tips of processes grown in KHC antisense conditions for the possibility that they are enriched in cytoskeletal structures but devoid of organelles.

**Development of Neurite Asymmetry in Null Cells**

During their initial growth, hippocampal neurons extend several processes; one of these ultimately differentiates into a long and slender axon while the remainder become dendrites. The commitment of a neurite to an axonal identity occurs when that process becomes significantly longer than the others (Goslin and Banker, 1989). In this study we find that hippocampal neurons that lack detectable KHC nevertheless become polarized, at least as assessed by morphologic criteria. Although process length is reduced, the cells nonetheless exhibit a distinctly asymmetric form, with one neurite far longer than any of the others. Appropriate markers will be necessary to establish the identity of the long process as an axon. Because treatment with KHC antisense oligonucleotides prevents the transport of GAP-43 and synapsin I into neurites, these commonly used axonal markers cannot be utilized. Other markers, like MAP-2 and \( \tau \) become segregated relatively late in the course of neuronal development in culture (Caceres et al., 1984; Kosik and Finch, 1987), too late for use in the present work.

Appropriate sorting of molecules must occur to create and maintain the molecular differences between axons and dendrites. The selective elongation of a single neurite is an early step in the development of neuronal polarity and represents the formation of an incipient axon. The results here suggest that the selective localization of proteins whose transport requires kinesin cannot be essential for the elaboration of an asymmetric array of neurites. GAP-43 appears to be among this class of proteins. The function of GAP-43, and its possible role in neurite outgrowth, remain controversial. Although it has been reported that GAP-43 antibodies delivered into neuroblastoma cells prevent neuritogenesis (Shea et al., 1991) and overexpression of GAP-43 accelerates neurite outgrowth in PC12 cells (Yankner et al., 1990), neurite outgrowth does occur normally in a PC12 line deficient in GAP-43 (Baetge and Hammang, 1991). GAP-43 normally accumulates in the axonal growth cones of cultured hippocampal neurons coincident with the emergence of the axon, suggesting that it could play a role in the establishment of polarity (Goslin and Banker, 1990). Since the inhibition of KHC expression resulted in the restriction of GAP-43 to the cell body, it is reasonable to suggest that GAP-43 need not be present in axons and their growth cones for axonal outgrowth and for the development of neurite asymmetry. There are, however, certain caveats to this interpretation. First, if the half-life of KHC is sufficiently long, then this motor protein may be present for some portion of the initial 24-h period. Some GAP-43 may have been transported into neurites during the first day in culture, before KHC levels had been completely reduced, but may have disappeared by 24 h, when we determined its localization. It is also possible that a small amount of GAP-43 was present in axons, but was below the level of detection by immunofluorescence.

The specific suppression of KHC expression by antisense...
techniques permits the assessment of the role of this motor and the transport of specific molecules in neuronal development. Retained neuronal function in the absence of the motor suggests that additional motors or other mechanisms may be operative in neurotropic elongation and the development of polarity. Which classes of organelles are transported by kinesin and exactly what degree of cellular integrity is retained in a KHCl-null state are compelling topics of further investigation.

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References

Baas, P. W., J. L. Deitch, M. M. Black, and G. A. Banker. 1988. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc. Natl. Acad. Sci. USA. 85:8335-8339.

Baas, P. W., J. L. Deitch, M. M. Black, and G. A. Banker. 1989. Changes in microtubule polarity orientation during the development of hippocampal neurons in culture. J. Cell Biol. 109:3085-3094.

Baetge, E. E., and J. P. Hammad. 1991. Neurite outgrowth in PC12 cells deficient in GAP-43. Neuron. 6:21-30.

Baas, P. W., J. M. Willard. 1987. Axonal transport of synapsin I-like proteins in rabbit retinal ganglion cells. J. Neurosci. 7:3732-3735.

Bartlett, W. P., and G. A. Banker. 1984. Electron microscopic studies of axonal and dendritic development by hippocampal neurons in culture. I. Cells which develop without intercellular contacts. J. Neurosci. 4:1944-1953.

Benowitz, L. J., V. E. Shashoua, and M. G. Yoon. 1981. Specific changes in sensitive protein kinase C substrate involved in rat brain phosphoinositide metabolism. J. Neurosci. 8:86-95.

Goslin, K., and G. A. Banker. 1989. Rapid changes in the distribution of GAP-43 correlate with the expression of neuronal polarity during development and under experimental conditions. J. Cell Biol. 110:1319-1331.

Goslin, K., and G. A. Banker. 1991. Raphe hippocampal neurons in low density culture. In Culturing Nerve Cells. G. Banker and K. Goslin, editors. The MIT Press, Cambridge, MA. 251-283.

Hall, D. H., and E. M. Hedgecock. 1991. Kinesin-related gene unc-104 is required for axonal transport of synaptic vesicles in C. elegans. Cell. 67:837-847.

Heideman, S., R. M. Landers, and M. A. Hamburger. 1981. Polarity orientation of axonal microtubules. J. Cell Biol. 91:661-665.

Hirokawa, N., R. Sat-Yoshitake, N. Kobayashi, K. K. Pfister, G. S. Bloom, and S. T. Brady. 1991. Kinesin associates with anterograde transport membrane organelles in axons. J. Cell Biol. 114:295-302.

Hollenbeck, P. J. 1989. The distribution, abundance and subcellular localization of kinesin. J. Cell Biol. 108:2335-2342.

Hollenbeck, P. J., and J. A. Swanson. 1990. Radial extension of microtubule tubulins supported by kinesin. Nature (Lond.). 346:864-866.

Hotani, H., and H. Miyamoto. 1990. Dynamic features of microtubules as visualized by dark-field microscopy. Adv. Biophys. 26:135-156.

Houliston, E., and R. P. Elison. 1991. Evidence for the involvement of microtubules, ER, and kinesin in the cortical rotation of fertilized frog eggs. J. Cell Biol. 114:1017-1028.

Ingold, A. L., S. A. Cohn, and J. M. Scholey. 1988. Inhibition of kinesin-driven microtubule motility by monoclonal antibodies to kinesin heavy chain. J. Cell Biol. 107:2657-2667.

Jahn, R., W. Schiebler, and P. Greengard. 1984. A quantitative dot-immunoassay for proteins using nitrocellulose membrane filters. Proc. Natl. Acad. Sci. USA. 81:1684-1687.

Kosik, K. S., K. S. Kosik, G. Lee, S. Standaert, L. Cohen-Gould, and L. McConlogue. 1991. Overexpression of tau in a non-neuronal cell induces long cellular processes. J. Cell Biol. 114:725-733.

Kosik, K. S., and E. A. Finch. 1987. MAP-2 and tau segregate into axonal and dendritic domains after their distribution of morphologically distinct neurites. An immunocytochemical study of cultured rat cerebrum. J. Neurosci. 7:3142-3156.

Kosik, K. S., L. D. Orecchio, B. J. Schnapp, H. Inouye, and R. L. Neve. 1990. The primary structure and analysis of the squid kinesin heavy chain. J. Biol. Chem. 265:3278-3283.

Kucik, D. F., E. L. Elson, and M. P. Sheetz. 1989. Forward transport of glyco-proteins on leading lamellipodia in locomoting cells. Nature (Lond.). 340:315-317.

Kuznetsov, S. A., E. A. Vaisberg, N. A. Shamina, N. N. Magvetova, V. X. Chernyak, and V. J. Gelfand. 1988. The quaternary structure of bovine brain kinesin. EMBO (Eur. Mol. Biol. Organ.) J. 7:353-356.

Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of the bacteriophage T4. Nature (Lond.). 227:680-685.

Navone, F., J. Nicolas, N. Horn-Booher, L. Sparks, H. Bernstein, G. McCaffrey, and R. D. Vale. 1992. Expression of the human kinesin heavy chain gene in C. elegans: interaction of the carboxy terminal domain with cytoplasmic microtubules. In press.

Otsuka, A. J., A. Jeyaprakash, J. Garcia-Anoveros, L. Z. Tang, G. Fisk, T. Harshthorne, R. Franco, and T. Born. 1991. The c. elegans unc-104 gene encodes a putative kinesin heavy chain-like protein. Dev. 6:113-122.

Pfenninger, K. H., and M. Mayle-Pfenninger. 1981. Lectin labeling of sprout- ing neurites. II. Relative movement and appearance of glycoconjugates during the development of peripheral axon. J. Cell Biol. 95:547-559.

Dr. Susan Schuh and Arlene Loewy. We thank J. Scholey for SUK4, M.

McCaffrey, and R. D. Vale. 1992. Expression of the human kinesin heavy chain gene in C. elegans: interaction of the carboxy terminal domain with cytoplasmic microtubules. In press.

Porter, E. M., J. M. Scholey, D. L. Stemple, G. P. A. Vigers, R. D. Vale, M. P. Sheetz, and R. J. McIntosh. 1987. Characterization of the microtubule movement produced by sea urchin egg kinesin. J. Biol. Chem. 262:2794-2802.

Rodov, V. I., F. K. Gyoeva, and V. J. Gelfand. 1991. Kinesin is responsible for centrifugal movement of pigment granules in melanophores. Proc. Natl. Acad. Sci. USA. 88:4956-4960.

Saxton, W. M., J. Hicks, L. S. Goldstein, and E. C. Raft. 1991. Kinesin heavy chain is essential for viability and neuromuscular functions in D. sophila, but mutants show no defects in mitosis. Cell. 64:1093-1102.

Scholey, J. M., E. M. Porter, P. M. Grissom, and R. J. McIntosh. 1987. Identification of kinesin in sea urchin eggs, and evidence for its localization in the mitotic spindle. Nature (Lond.). 318:483-486.

Shea, T. B., N. I. Perrone-Bizzozero, M. L. Beermann, and L. I. Benowitz. 1991. Phospholipid-mediated delivery of anti-GAP-43 antibodies into neuronal cell processes prevents cells from neurogenesis. J. Neurosci. 11:1685-1690.

Sky, P. J. H., and I. Virag. 1989. Posttranslational modifications and expression of the squid kinesin heavy chain. In Kinesin Structure, Function and Evolution. H. J. Jaffe, ed. Cold Spring Harbor Laboratory Press, New York. 199-228.

Skene, J. H., and P. J. H. Sky. 1989. The C. elegans unc-104 gene is required for axonal transport of synaptic vesicles in C. elegans. J. Cell Biol. 108:613-624.

Skelton, C. J., and H. N. Millard. 1981. Changes in axonally transported proteins during axon regeneration in toad retinal ganglion cell. J. Cell Biol. 89:86-95.

Ferreira et al. Kinesin Antisense
Small, R. K., M. Blank, R. Ghez, and K. H. Pfenninger. 1984. Components of the plasma membrane of growing axons. II. Diffusion of membrane protein complexes. J. Cell Biol. 98:1434–1443.

Steuer, E. R., L. Wordeman, T. A. Schroer, and M. P. Sheetz. 1990. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. Nature (Lond.) 345:266–268.

Tanaka, E. M., and M. W. Kirschner. 1991. Microtubule behavior in the growth cones of living neurons during axon elongation. J. Cell Biol. 115:345–363.

Urritia, R., M. A. McNiven, J. P. Albanesi, D. B. Murphy, and B. Kachar. 1991. Purified kinesin promotes vesicle motility and induces active sliding between microtubules in vitro. Proc. Natl. Acad. Sci. USA. 88:6701–6705.

Vale, R. D., T. S. Reese, and M. P. Sheetz. 1985a. Identification of a novel force generating protein, kinesin, involved in microtubule-based motility. Cell. 42:39–50.

Vale, R. D., B. J. Schnapp, T. Mitchison, E. Steuer, T. S. Reese, and M. P. Sheetz. 1985b. Different axoplasmic proteins generate movement in opposite directions along microtubules in vitro. Cell. 43:623–632.

Valtorta, F., A. Villa, R. Jahn, P. DeCamilli, P. Greengard, and B. Ceccarelli. 1988. Localization of synapsin I at the frog neuromuscular junction. Neuroscience. 24:593–603.

Wright, B. D., J. H. Henson, K. P. Wedaman, P. J. Willy, J. N. Morand, and J. M. Scholey. 1991. Subcellular localization and sequence of sea urchin kinesin heavy chain: evidence for its association with membranes in the mitotic spindle apparatus and interphase cytoplasm. J. Cell Biol. 113:817–833.

Yang, J. T., R. A. Laymon, and L. S. B. Goldstein. 1989. A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analyses. Cell. 56:879–889.

Yankner, B. A., L. I. Benowitz, L. Villa-Komaroff, and R. L. Neve. 1990. Transfection of PC12 cells with the human GAP-43 gene: effects on neurite outgrowth and regeneration. Mol. Brain Res. 7:39–44.