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Cloning of a big tau microtubule-associated protein characteristic of the peripheral nervous system

(sensory ganglia/sympathetic ganglia/central nervous system/alternative mRNA splicing)

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ABSTRACT **Microtubule-associated protein tau consists in brain of a series of isoforms of 48- to 67-kDa apparent molecular mass that are encoded by mRNAs of approximately 6 kilobases (kb) and that are generated from a single gene by alternative splicing. Previously, a tau-like protein of 110-kDa apparent molecular mass was described in peripheral ganglia and in peripheral neuronlike cell lines. We now report the cloning and sequencing of a rat cDNA encoding this big tau. The corresponding protein contains sequence identical to the longest of the previously cloned small tau isoforms but with an additional 254 amino acid insert in the amino-terminal half. Big tau is produced from an 8-kb mRNA generated by alternative splicing from the same gene that encodes small tau. Production of big tau from the cloned sequence gives a protein of 110-kDa apparent molecular mass that aligns on SDS/PAGE with big tau protein extracted from peripheral ganglia. RNA blots show that in peripheral ganglia from adult rats only the 8-kb mRNA band corresponding to big tau is found, whereas in ganglia from newborn rats both 6- and 8-kb tau mRNA bands are found. In tissues from the central nervous system only the 6-kb mRNA band can be detected. Big tau protein is therefore produced specifically in the peripheral nervous system, and it will be interesting to see whether further molecular differences between the two major divisions of the vertebrate nervous system will be discovered.**

Brain microtubules contain a variety of proteins that copurify with tubulin during repeated cycles of assembly and disassembly (1). Microtubule-associated proteins promote the assembly and stability of microtubules and play an important role in the determination of neuronal morphology and plasticity (2–4). Among microtubule-associated proteins tau constitutes a family of neuronal proteins that are largely confined to axons (for a recent review, see ref. 5). Tau in adult human brain is found as a set of six related proteins 352 to 441 amino acids in length with apparent molecular masses of 48–67 kDa (6, 7) that are generated from a single gene by alternative mRNA splicing (8–10). They differ by the presence of three or four tandem repeats located in the carboxyl-terminal half and by the presence or absence of 29 or 58 amino acid inserts located in the amino-terminal half. The tandem repeats constitute the microtubule-binding domains of tau (11–14), while the function of the amino-terminal inserts is unknown. Tau isoforms with different inserts are expressed in a stage- and cell type-specific manner. Thus, the three-repeat isoform without amino-terminal inserts is expressed in fetal human brain, whereas all six isoforms are expressed in adult human brain (6, 8). Moreover, in adult human hippocampus, transcripts encoding isoforms with three repeats are present in granule and pyramidal cells, whereas transcripts encoding isoforms with four repeats are found only in pyramidal cells (8).

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Traditionally, the vertebrate nervous system has on anatomical grounds been subdivided into central and peripheral divisions. No corresponding differences at a molecular level have been established. However, ganglia in the peripheral nervous system and cell lines with peripheral neuron-like characteristics have been reported to contain a protein of apparent molecular mass 110 kDa that reacts with anti-tau antibodies (15–20). The presence of the 110-kDa protein correlates with that of an 8-kilobase (kb) mRNA recognized by probes derived from tau cDNA clones (18–20), whereas the brain tau isoforms are encoded by mRNAs of about 6 kb. These observations suggest that the 110-kDa tau-like protein might be a candidate for a protein produced specifically in the peripheral nervous system.

Here we report the cloning and sequencing of a rat cDNA for this big tau.* The encoded protein of 686 amino acids is expressed from the same gene as the small tau isoforms but differs from small tau proteins by an insert of 254 amino acids located in the amino-terminal half. The corresponding 8-kb mRNA is found in nerve cells in sensory and sympathetic ganglia but not in brain or spinal cord. The expression pattern is developmentally regulated in peripheral ganglia, since in newborn animals both 6- and 8-kb mRNAs are present, whereas in adults only the 8-kb mRNA can be detected. Tau mRNA is thus differently spliced in the central and peripheral divisions of the vertebrate nervous system, and big tau provides an example of a protein produced specifically in the peripheral division.

MATERIALS AND METHODS

Isolation of a cDNA Clone Encoding Big tau Protein. A 1029-base-pair (bp) *Sac* I/*Nhe* I fragment from the coding region of the human tau cDNA clone 40 (6) was labeled by the random primer technique (21) and used to screen a rat PC12 cDNA library in λgt10 (obtained from Clontech) at high stringency. Eight positive clones were isolated from 300,000 plaques, and the longest (clone PC12-8) was subcloned in M13mp18 and sequenced by using the chain termination method (22) with synthetic oligonucleotides as primers.

RNA Blot Analysis and *in Situ* Hybridization. Tissues were dissected from either 2-day-old or adult Sprague–Dawley rats and immediately frozen on dry ice.

RNA extractions, hybridizations, and washings were performed exactly as described (23). A probe specific for big tau mRNA was produced from clone PC12-8 (nucleotides 398–764) by using the polymerase chain reaction, purified by gel electrophoresis, and labeled with [α-^{32}P]dATP by the random primer technique (22). A general rat tau probe was produced by labeling the *Eco*RI insert of clone PC12-8 by the random primer technique. After dehybridization some blots were rehybridized with a mouse β-actin cDNA clone labeled by the random primer technique (23). The 0.24- to 9.5-kb RNA

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M84156).

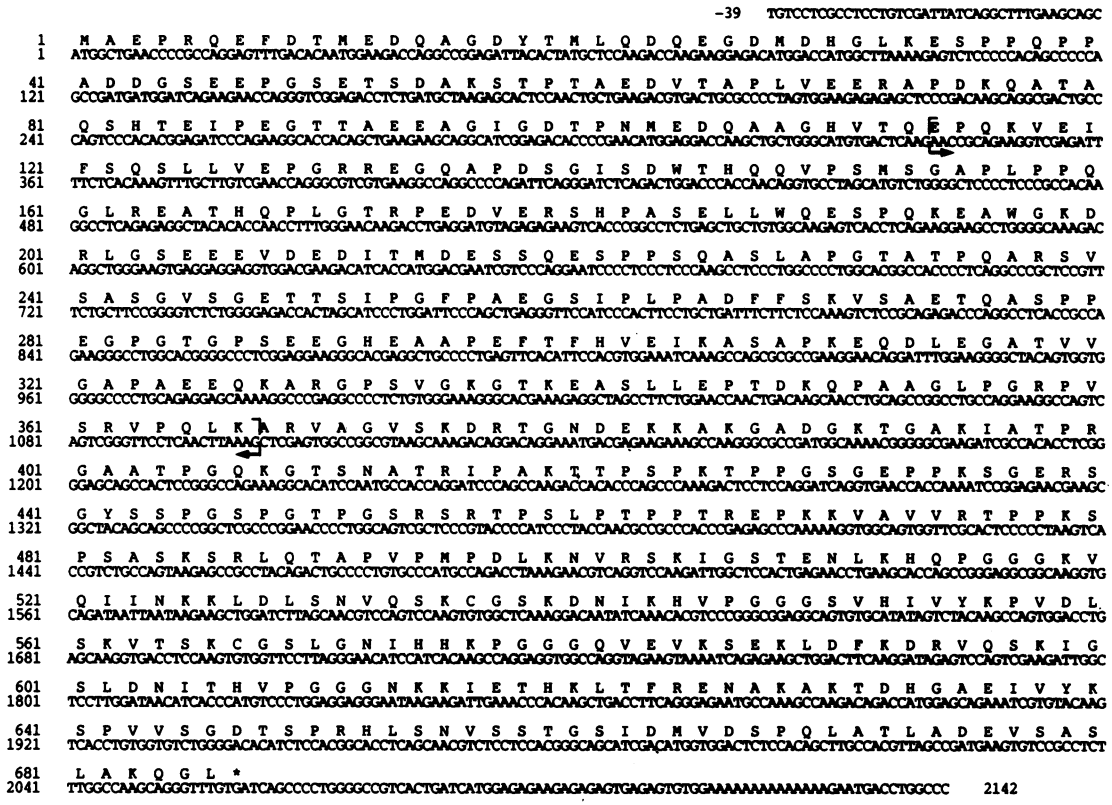

FIG. 1. Nucleotide and predicted amino acid sequences of rat big tau. The sequences differ from known tau sequences by the bracketed 762 nucleotides, which encode a 254 amino acid insert. Nucleotides are numbered in the 5′-to-3′ direction, starting with the first nucleotide of the initiating codon; the nucleotides 5′ to residue 1 are indicated by negative numbers.

ladder (Bethesda Research Laboratories) served as size marker.

In situ hybridization was performed by using a 45-mer oligonucleotide specific for big tau (corresponding to nucleotides 887–931 of clone PC12-8) in the anti-mRNA sense orientation. A sense oligonucleotide served as a control. Probe labelings with deoxyadenosine 5′-[α-[^{32}S]thio]triphosphate and hybridizations were performed as described (8). The final wash was done in 1× SSC (150 mM NaCl/15 mM sodium citrate, pH 7) at 42°C overnight, followed by dipping in emulsion and developing after 2 weeks.

Expression of Big tau Protein in *Escherichia coli* and Extraction from Rat Tissues. For the expression of big tau protein, site-directed mutagenesis was used to introduce a *Nde* I site in the context of the initiator codon of cDNA clone PC12-8. The resulting *Nde* I/*Eco*RI fragment was subcloned in the expression plasmid pRK172 (24) under control of the phage T7 RNA polymerase promoter. Transformation of *E. coli* BL21(DE3) cells (25), induction of expression, and harvesting of the expressed protein were done as described (7). The equivalent of 5 μl of bacterial culture was electrophoresed on an SDS/10% polyacrylamide gel.

Big tau protein was extracted from adult rat trigeminal ganglia by using a modification of the method described for cultured cells (18). Individual ganglia were sonicated in 200 μl of 25 mM sodium phosphate buffer, pH 7.4, containing 0.4 M NaCl, 0.5% SDS, and phenylmethylsulfonyl fluoride at 1 mg/ml, and the solution was boiled for 3 min and centrifuged. Forty microliters of the supernatant was electrophoresed on an SDS/10% polyacrylamide gel.

Proteins were electrophoretically transferred to nitrocellulose (BA83, Schleicher & Schuell) and immunoblotted, using the anti-tau antiserum 135 (6) and the ABC Vectastain system (Vector Laboratories).

RESULTS

In a search for cDNA clones encoding big tau, a library prepared from the rat pheochromocytoma cell line PC12 was

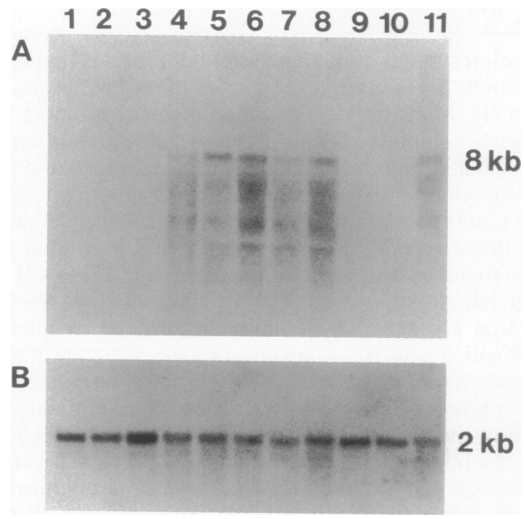

FIG. 2. Blot analysis of RNA from central and peripheral rat nervous system tissues, with big tau-specific DNA (*A*) or β-actin DNA (*B*) used as a probe. Lanes 1–3, 9, and 10 contained 3 μg of poly(A)$^+$ RNA, whereas lanes 4–8 and 11 contained 30 μg of total RNA. Lanes: 1, adult cerebral cortex; 2, adult cerebellum; 3, adult spinal cord; 4, adult dorsal root ganglion; 5, newborn trigeminal ganglion; 6, adult trigeminal ganglion; 7, newborn superior cervical ganglion; 8, adult superior cervical ganglion; 9, whole newborn brain; 10, whole adult brain; 11, PC12 cells. An 8-kb big tau band is detected in lanes 4–8 and 11 but not in lanes 1–3, 9, and 10, whereas the 2-kb actin control band is present in all lanes. The smear below the 8-kb band in *A* probably represents partial degradation of the poly(A)$^+$ RNA.

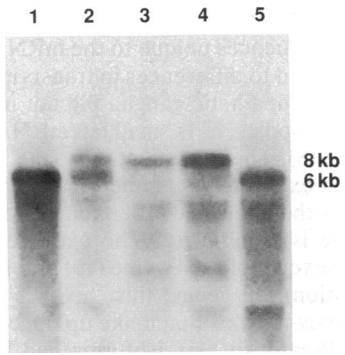

FIG. 3. Blot analysis of RNA from central and peripheral rat nervous system tissues, with clone PC12-8 used as a general tau probe. All lanes contained 3 μg of poly(A)$^+$ RNA. Lanes: 1, whole adult brain; 2, newborn trigeminal ganglion; 3, adult trigeminal ganglion; 4, adult dorsal root ganglion; and 5, adult spinal cord.

screened. A full-length cDNA sequence was isolated; it encodes a predicted 686 amino acid protein with a molecular weight of 71,783. This protein contains sequence identical to that of the longest of the small tau isoforms from rat brain (26, 27) but with an additional 254 amino acid insertion in the amino-terminal half (Fig. 1). The identity in sequence at the DNA level indicates that big tau is encoded by the same gene as the small tau isoforms and, like them, is generated by alternative mRNA splicing.

The expression pattern of big tau mRNA was studied in the central nervous system and in neuronal and nonneuronal peripheral rat tissues by RNA blotting using a probe specific for the inserted sequence (Fig. 2). An 8-kb mRNA band was observed in dorsal root ganglia, trigeminal ganglia, and sympathetic ganglia from both newborn and adult rats. No transcripts could be detected in brain or spinal cord (Fig. 2) or in any of the nonneuronal peripheral tissues examined (data not shown). In PC12 cells a band of 8-kb mRNA was observed. When a general tau probe was used, only a 6-kb mRNA band was observed throughout the central nervous system of newborn and adult rats, while only the 8-kb mRNA band was seen in adult sensory and sympathetic ganglia (Fig. 3). By contrast, in peripheral ganglia from newborn animals, both the 6- and 8-kb mRNA bands were present (Fig. 3). Both 6- and 8-kb bands were also seen in PC12 cells (data not shown). By *in situ* hybridization, using a probe specific for the sequence encoding the big tau insert, big tau mRNA was localized in nerve cells in adult sensory and sympathetic ganglia (Fig. 4). All nerve cell bodies appeared to be hybridization-positive. When *in situ* hybridization with the big tau probe was performed on sections taken throughout the adult rat brain, spinal cord, and small intestine, no specific signal was observed (data not shown). It thus seems likely that the big tau protein detected in spinal cord (20) is produced in dorsal root ganglia.

Big tau was produced from the cloned sequence in *E. coli* by using an expression plasmid under the control of the T7 RNA polymerase promoter. It was detected by SDS/PAGE and identified by immunoblotting. Recombinant big tau ran with an apparent molecular mass of 110 kDa and aligned on gels with big tau protein extracted from adult rat trigeminal ganglia (Fig. 5).

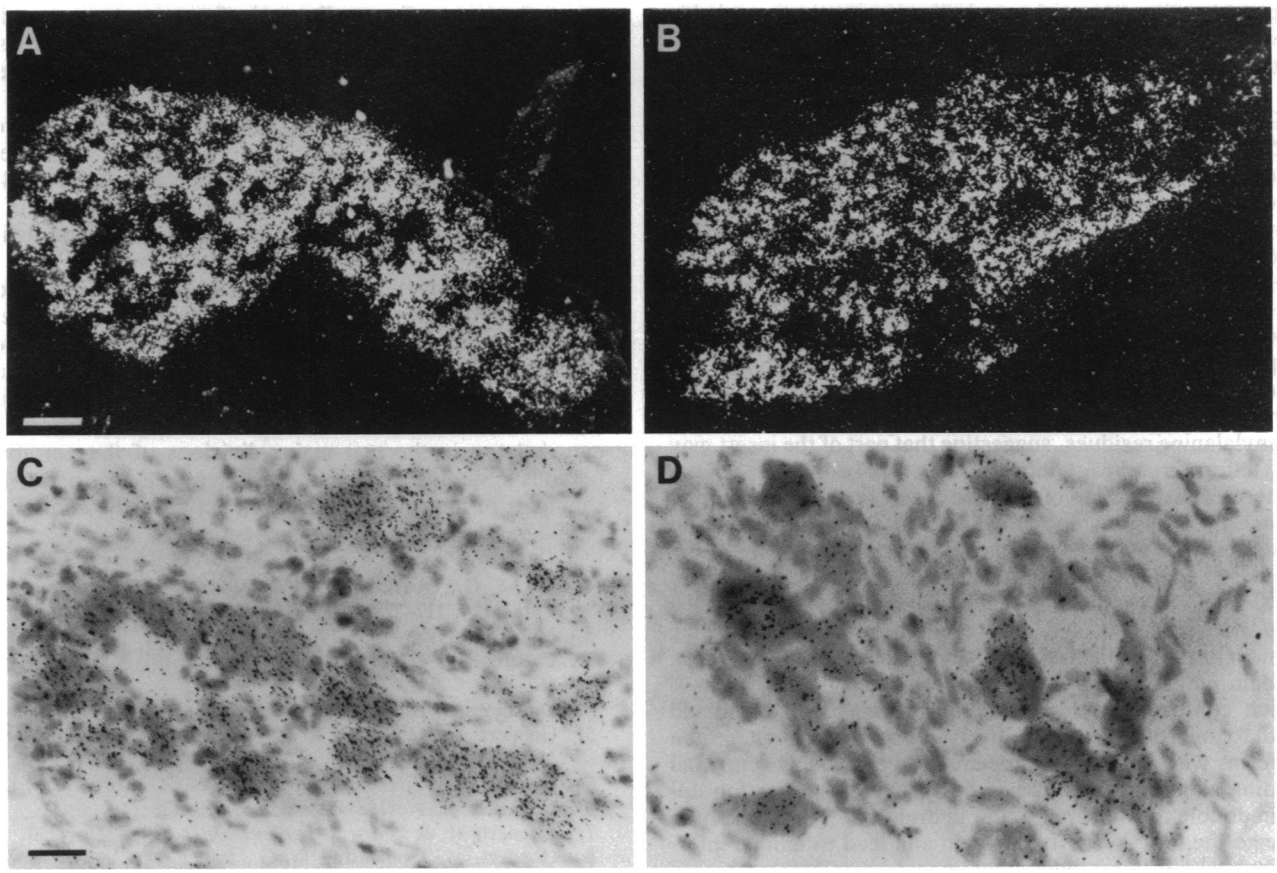

FIG. 4. Cellular localization of big tau mRNA in adult rat sensory and sympathetic ganglia. (*A* and *B*) Dark-field photomicrographs of dorsal root ganglion (*A*) and superior cervical ganglion (*B*) sections. (*C* and *D*) Bright-field photomicrographs of dorsal root ganglion (*C*) and trigeminal ganglion (*D*) sections. The tissue sections were hybridized with a big tau-specific probe in the anti-mRNA sense orientation. There is specific labeling of the large cell bodies corresponding to neurons but not of the smaller glial cells. Only background labeling was observed with a probe in the mRNA sense orientation (data not shown). (Scale bars, 105 μm for *A* and *B*, 25 μm for *C* and *D*.)

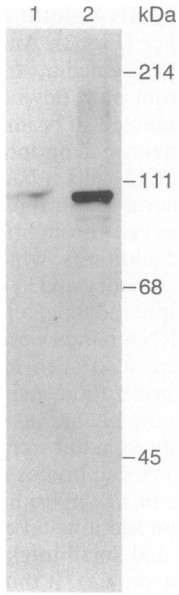

FIG. 5. Comparison of native and recombinant big tau proteins. Following electrophoresis on an SDS/10% polyacrylamide gel, tau proteins were visualized by immunoblotting. Lane 1, big tau protein extracted from an adult rat trigeminal ganglion. Lane 2, recombinant big tau protein. The numbers on the right indicate positions of the molecular mass markers.

DISCUSSION

The big tau protein we have characterized in sequence, apparent molecular weight, and other properties is probably identical with the high molecular weight protein reactive with anti-tau antibodies whose existence was previously reported in sensory ganglia, sympathetic ganglia, and PC12 cells (15–20). It differs from all previously cloned tau isoforms by a 254 amino acid insert located in the amino-terminal half. The position of the insert corresponds to a splice junction in bovine (10), human, and rat small tau mRNAs (unpublished observation), indicating that it results through alternative mRNA splicing from the tau gene. Moreover, sequencing of rat genomic clones has shown that the 254 amino acid insert is encoded by a single exon (unpublished observation). Comparison of the inserted sequence with known protein sequences failed to reveal any significant homology. The inserted sequence contains three tryptophan residues, in contrast with small tau (6), which contains none, and also six phenylalanine residues, suggesting that part of the insert may fold to form a compact domain. As in small tau (6) the overall pattern of distribution of charged residues shows a predominance of acidic residues towards the amino terminus and basic residues towards the carboxyl terminus.

Like small tau isoforms (5), big tau is anomalously retarded on SDS/PAGE, indicating that it also may have an extended conformation. Tau is composed of a microtubule-binding domain located in the carboxyl-terminal half and a projection domain consisting of the amino-terminal half (5). Big tau, which contains the entire tubulin-binding domain, should bind to microtubules with the same affinity as other tau isoforms with four tandem repeats. It would thus occupy tau binding sites on microtubules but provide a projection domain double the size that found in brain tau. This is likely to change the function of tau protein and lead to unique interactions with other axonal components.

Big tau is encoded by an 8-kb mRNA that is derived from the same gene as the set of 6-kb mRNAs that encodes small tau isoforms. The 8-kb and 6-kb mRNAs must therefore have different lengths of untranslated RNA, approximately 6 kb for big tau mRNA and approximately 4.7 kb for the mRNAs of other tau isoforms; this implies the existence of 5′ and (or) 3′ untranslated sequences unique to the mRNA encoding big tau, possibly related to differences in transcriptional control. In sensory and sympathetic ganglia big tau mRNA is localized to nerve cell bodies, as is small tau mRNA in the central nervous system (8). The identification of the 254 amino acid insert in big tau extends the number of known tau isoforms and implies that the published (10) exon/intron organization of the tau gene is incomplete. The exon reported here is spliced in after exon 4 of the bovine (10) and human (unpublished observation) tau genes; this "exon 5" is by far the largest of the known exons that make up the coding region of the tau gene. Previously, six isoforms had been identified through cDNA cloning and expression as the major adult human brain tau proteins (6, 7). They differ by the number of tandemly repeated microtubule-binding domains (3 or 4) and the presence or absence of an insert (29 or 58 amino acids) in the amino-terminal half. If the same exon combinations occur in presence of the 254 amino acid insert this will double the number of known tau isoforms. Further studies are needed to provide a detailed description of the tau isoform composition in sensory and sympathetic ganglia and in particular to show whether the big tau insert occurs with combinations of small tau exons other than that cloned in this study.

As shown here, 8-kb big tau mRNA is found in sensory and sympathetic ganglia in newborn and adult rats but not in brain or spinal cord of either newborn or adult rats. By contrast, 6-kb small tau mRNA is found in brain and spinal cord in newborn and adult rats. However, the 6-kb mRNA is also present in sensory and sympathetic ganglia in newborn but not adult rats. It follows that in developing sensory and sympathetic ganglia small tau isoforms are expressed in conjunction with big tau-containing isoform(s), whereas in adult ganglia only big tau-containing isoform(s) are present. These results thus add a further level of complexity to the developmental expression of tau isoforms in the nervous system. PC12 cells, derived from a tumor of the adrenal medulla, also contain the 6-kb small tau mRNA and the 8-kb big tau mRNA. However, as shown before (23, 28), tau mRNAs could not be detected in the adrenal gland or in a variety of other nonneuronal peripheral tissues.

Thus, big tau is an isoform that is specifically expressed in the peripheral nervous system, implying the existence of alternative splicing between peripheral and central nervous systems. The topographical division into peripheral and central nervous systems is thus paralleled by molecular differences that may result in a distinct organization of the cytoskeleton in the two major divisions of the vertebrate nervous system. It will be interesting to see whether further examples of molecular differences between the central and peripheral nervous systems emerge.

1. Wiche, G., Oberkanins, C. & Himmler, A. (1991) *Int. Rev. Cytol.* **124**, 217–273.
2. Matus, A. (1988) *Annu. Rev. Neurosci.* **11**, 29–44.
3. Caceres, A. & Kosik, K. S. (1990) *Nature (London)* **343**, 461–463.
4. Dinsmore, J. H. & Solomon, F. (1991) *Cell* **64**, 817–826.
5. Goedert, M., Crowther, R. A. & Garner, C. C. (1991) *Trends Neurosci.* **14**, 193–199.
6. Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D. & Crowther, R. A. (1989) *Neuron* **3**, 519–526.
7. Goedert, M. & Jakes, R. (1990) *EMBO J.* **9**, 4225–4230.
8. Goedert, M., Spillantini, M. G., Potier, M. C., Ulrich, J. & Crowther, R. A. (1989) *EMBO J.* **8**, 393–399.
9. Himmler, A., Drechsel, D., Kirschner, M. W. & Martin, D. W. (1989) *Mol. Cell. Biol.* **9**, 1381–1388.
10. Himmler, A. (1989) *Mol. Cell. Biol.* **9**, 1389–1396.
11. Aizawa, H., Kawasaki, H., Murofushi, K., Kotani, S., Suzuki, K. & Sakai, H. (1989) *J. Biol. Chem.* **264**, 5885–5890.

12. Ennulat, D. J., Liem, R. K. M., Hashim, G. A. & Shelanski, M. J. (1989) *J. Biol. Chem.* **264,** 5327–5330.
13. Lee, G., Neve, R. L. & Kosik, K. S. (1989) *Neuron* **2,** 1615–1624.
14. Butner, K. A. & Kirschner, M. W. (1991) *J. Cell Biol.* **115,** 717–730.
15. Drubin, D. G., Feinstein, S. C., Shooter, E. M. & Kirschner, M. W. (1985) *J. Cell Biol.* **101,** 1799–1807.
16. Peng, J., Binder, L. I. & Black, M. M. (1986) *J. Cell Biol.* **102,** 2532–2542.
17. Drubin, D., Kobayashi, S. & Kirschner, M. W. (1986) *Ann. N.Y. Acad. Sci.* **466,** 257–268.
18. Drubin, D., Kobayashi, S., Kellogg, D. & Kirschner, M. W. (1988) *J. Cell Biol.* **106,** 1583–1591.
19. Oblinger, M. M., Argasinski, A., Wong, J. & Kosik, K. S. (1991) *J. Neurosci.* **11,** 2453–2459.
20. Georgieff, I. S., Liem, R. K. H., Mellado, W., Nunez, J. & Shelanski, M. L. (1991) *J. Cell Sci.* **100,** 55–60.
21. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
22. Sanger, F., Nicklen, S. & Coulson, A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
23. Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E. & Klug, A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4051–4055.
24. McLeod, M., Stein, M. & Beach, D. (1987) *EMBO J.* **6,** 729–734.
25. Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990) *Methods Enzymol.* **185,** 60–89.
26. Kanai, Y., Tsakemura, R., Oshima, T., Mori, H., Ihara, Y., Yanagisawa, M., Masaki, T. & Hirokawa, N. (1989) *J. Cell Biol.* **109,** 1173–1184.
27. Kosik, K. S., Orecchio, L. D., Bakalis, S. & Neve, R. L. (1989) *Neuron* **2,** 1389–1397.
28. Neve, R. L., Harris, P., Kosik, K. S., Kurnit, D. M. & Donlon, T. A. (1986) *Mol. Brain Res.* **1,** 271–280.