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Tau Protein and Neurodegeneration

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Abstract

Many of the human neurodegenerative conditions involve a reorganization of the neuronal cytoskeleton. The way in which the cytoskeleton is reorganized may provide a clue to the nature of the insult causing the neurodegeneration. The most common of these conditions is Alzheimer's disease, in which microtubules are lost from neurites that fill up with filamentous structures. One component of the filamentous structures is the microtubule-associated protein (MAP), tau. The tau protein is the product of a single gene expressed predominantly in neurons. The tau gene undergoes complex alternative splicing that is regulated both by development, and by the particular neuronal cell population in which it is expressed. Tau protein can be further modified, following its translation by phosphorylation at several sites. Much of the recent interest in the transition of tau to an abnormal state within a tangle-bearing neuron has focused on phosphorylation. A group of proteins that migrate slightly more slowly than tau, designated PHF-tau, are found in regions of the Alzheimer brain rich in dystrophic neurites, are hyperphosphorylated, fail to bind to microtubules, have distinct solubility properties, and can be derived from fractions of paired helical filaments (PHF).

Index Entries: Tau; neurodegeneration; phosphorylation; Alzheimer's disease; paired helical filaments; microtubule-associated proteins.

Introduction

Many of the conditions grouped under the broad designation of "neurodegenerative" involve a reorganization of the cytoskeleton. These conditions are often associated with intraneuronal inclusions, which in their various forms often consist of cytoskeletal elements and the protein ubiquitin. One of the most common of these inclusions is the neurofibrillary tangle. This structure is the subject of numerous studies, because it is one of the classical hallmarks of Alzheimer's disease. The principal ultrastructural component of the neurofibrillary tangle is the paired helical filament (PHF). Although other cellular debris is observed by electron microscopy within the tangle, the composition and pathogenesis of the PHF represents the most commonly pursued object of study. Neurofibrillary degeneration is very likely a complex pathobiological process that traps PHF within the cellular milieu as part of the neurodegenerative process.

Recently a number of insights concerning the molecular composition and pathogenesis of the neurofibrillary tangle have appeared. These studies have culminated in the observation that the microtubule-associated protein (MAP) tau, is the major, if not the exclusive, constituent of the Alzheimer PHF (Greenberg and Davies, 1990; Lee et al., 1991). To approach the significance of tau protein as a component of neurofibrillary degeneration requires placing tau in its context among the diverse family of MAPs. Within the MAP family are proteins with highly divergent sequences derived from multiple genes that all share the property of binding to microtubules. Individual MAPs attain further diversity by complex splicing reactions that include and exclude specific exons in an array of patterns and phosphorylation events at multiple sites within these molecules. Although the in vivo functions of these proteins remain unclear, in vitro they have the property of promoting the polymerization of microtubules (Weingarten et al., 1975). In the context of current models of dynamic instability (Mitchison and Kirschner, 1984), MAPs appear to decrease the frequency of transition between the growing and shrinking phases of microtubules in vitro (Horio and Hotani, 1986), presumably by reducing microtubule instability (Bre and Karsenti, 1990). Since microtubules have a role in the generation and maintenance of cell shape, the modulation of the dynamic behavior of microtubules by MAPs may contribute to creating the diversity of spatial arrays found among cells, particularly neurons. Another crucial role of microtubules is their vectorial role in the guidance of organelle translocation. A class of proteins more loosely asociated with microtubules serve as motors to guide organelles toward their destinations (McIntosh and Porter, 1989). Because these mechanoenzymes, kinesin and dynein, are defined by whether they move organelles toward the "plus" or the "minus" end of the microtubules, the orientation of the microtubules in various parts of the neuron is crucial to assure correct targeting of organelles (Black and Baas, 1989). The maintenance of targeted delivery of organelles certainly requires stably polymerized microtubules. Although a number of modifications of tubulin, including acetylation and detyrosination, are associated with microtubule stability, MAPs are likely to have a very direct role in the stabilization of microtubules.

The Normal Tau Molecule

The MAP tau is the product of a single gene (Neve et al., 1986) that undergoes complex alternative splicing (Lee et al., 1988; Himmler, 1989). It has been mapped to the long arm of chromo-some 17 (Neve et al., 1986) and located on a gen-etic linkage map of the chromosome (Haines et al., 1990). The cloning and sequencing of tau cDNAs have suggested certain structure–function relationships. The most apparent was the finding of three imperfectly repeated sequences, each of 31–32 amino acid, near the carboxy terminus of tau, which have the property of microtubule binding (Lee et al., 1989; Himmler et al., 1989). This finding derived from

in vitro studies in which tau deletion constructs lacking portions of the carboxy terminus were expressed and assayed for their ability to bind to microtubules. Similar binding properties have been attributed to these sequences based on the use of complementary synthetic peptides (Aizawa et al., 1989; Ennulat et al., 1989). To approximate more closely the in vivo behavior of tau, transfection of tau cDNAs in mammalian cells were used. Investigators who have expressed various tau sequences in mouse fibroblast L-cells (Kanai et al., 1989) and a variety of other cell types (Lewis et al., 1989) have observed that some of their constructs result in the bundling of microtubules within the cytoplasm. It is unresolved what the minimal tau sequences required for bundling are, however, some of the variability in the induction of bundling observed by different investigators is very likely a result of distinct effects of tau expression in different host cells.

Tau is synthesized in neurons (Kosik et al., 1989) and abundant within the axonal compartment (Binder et al., 1985; Peng et al., 1986; Kowall and Kosik, 1987; Brion et al., 1988). Another neuronal MAP, MAP2, is found in the somatodendritic compartment of neurons, and thus has the complementary cytological topography to tau. Although microtubules are present throughout the neuron, they associate with distinct MAPs, depending on their setting in an axonal or dendritic compartment. How this compartmentation arises is unknown. The sorting of distinct tubulin isotypes with differential affinities for the MAPs is one testable way by which MAP compartmentation could arise. It should also be appreciated that the rules of the cellular localization of the MAPs are not absolute. For instance, although tau is considered primarily a neuronal protein, it may also be found in other cell types that bear no obvious relationship to mammalian neurons. Proteins immunoreactive with several different tau monoclonal antibodies were found in the erythrocyte marginal band of the dogfish (Sanchez et al., 1990) and the chicken (Murphy and Wallis, 1985). The unique spatial and mechanical features of the marginal band, with its microtubules running over great distances in an array that is parallel to the plasma membrane, may require the specific properties of a tau-like MAP. Tau immunoreactivity has even been reported in cotton (*Gossypium hirsutum*) suspension cells (Seagull et al., 1990). Perhaps a particular MAP appears where a cell has a specific problem to solve, rather than as an absolute defining property of a cell type or a cell lineage. Tau-like proteins may occur in settings where parallel bundles of microtubules are required for cell function. The degree to which these and other tau-crossreactive proteins bear sequence homology to mammalian neuronal tau must be determined.

The Cell Biology and Pathobiology of Tau

The observation that MAP2 and tau are compartmented in neurons has prompted studies that have sought to explore a role for these proteins in the generation of the unique morphologies of axons and dendrites. In the case of tau protein there is some evidence to support such a role. The minor neurites elaborated by neurons in culture just after plating are all tau-immunoreactive; even after a single neurite undergoes elongation to form an axon, all the neurites remain tau-immunoreactive. Although tau is eventually sorted selectively to the axon in rat cerebrocortical cultures, it does not appear localized there until well after the neurite attains an axonal morphology (Kosik and Finch, 1987). This immunocytochemical localization may cast doubts on the role of tau protein in the generation of polarity, but another experimental approach has suggested that tau could function coordinately with other cellular events to create asymmetric neuronal morphologies. This approach utilized tau antisense oligonucleotides added to neuronal cell cultures in order to inhibit specifically the elaboration of an elongated, nontapering, axonlike structure (Caceres and Kosik, 1990). When tau antisense

oligonucleotides were administered to more mature cultures, there was a specific loss of neurites with an axonlike morphology, while dendritelike neurites continued to grow and increased in complexity (Caceres et al., in press). Furthermore, the expression of tau protein in a foreign host cell results in the elaboration of long, thin, relatively unbranched processes that bear a morphological similarity to axons (Knops et al., 1991). These experiments utilized a baculovirus vector to express tau protein in Sf9 cells, a type of cell derived from the moth ovary, that does not ordinarily express tau.

Given the possibility of a role for tau in the generation of an axonal morphology, it is of interest that recent immunohistochemical observations in the Alzheimer brain suggest a loss of resolution of many neurites as axons or dendrites. These studies used tau antibodies on sections of Alzheimer brain tissue to describe the neurofibrillary lesions. In addition to neurofibrillary tangles, tau antibodies label a population of neurites that range from having a nearly normal, threadlike appearance to thickened curly fibers (Kowall and Kosik, 1987; Braak et al., 1986; Braak and Braak, 1988). Among the nearly normal appearing neurites labeled with the tau antibody are pyramidal-cell apical dendrites (McKee et al., 1989). The immunolabeling of tau within the apical dendrite represents a distinctly aberrant cyto-logical localization of the tau protein. It is likely that these affected neurites have also lost their MAP2 immunoreactivity. Tau-reactive dystrophic neurites also affect axonal populations, and some of these neurites may even represent new sprouts (Ihara, 1988).

Dystrophic neurites are observed both around senile plaques and unassociated with plaques in the neuropil. The importance of these neurites has been underscored by the general agreement that tau-reactive neurites are critical neuropathological correlates of the clinical dementia of Alzheimer's disease (Barcikowska et al., 1989; Crystal et al., 1988; Arai et al., 1990; McKee et al., in press). The finding of β-amyloid protein immunoreactivity in the brain, although a neccessary lesion, is alone insufficient for the diagnosis of Alzheimer's disease. The high frequency of amyloid deposition in normal aging accounts for the poor correlation to clinical dementia. The predominant cytoskeletal feature of dystrophic neurites are processes that have either lost or destabilized their microtubules and have become filled with highly phosphorylated filamentous structures. A number of experimental systems address different aspects of this type of cytoskeketal reorganization.

PC12 cells have been used to show a correlation of neurite outgrowth to the induction of tau protein (Drubin et al., 1985) and tau mRNA (Drubin et al., 1988). Consistent with this observation are the reports that tau expression increases rapidly during neural development at a time when axonal outgrowth occurs, and then decreases as the animal matures (Couchie et al., 1988; Mangin et al., 1989). However, increased tau synthesis in the adult rat dorsal root ganglion was not observed during axonal regeneration after a crush injury to the sciatic nerve (Oblinger et al., in press). Axonal outgrowth in a regenerative setting may not, therefore, represent an identical phenomenon with regard to the organization of the microtubules as axonal outgrowth in a developmental context. The features that make the regenerative response distinct from development are nowhere more clear than in the aberrant response to close axotomy in the lamprey, discussed below. The aberrant features of this stereotypic regenerative response resemble some aspects of the neurofibrillary degeneration of Alzheimer's disease.

A system in which injury results in the assumption of axonal properties within dendrites and neuronal sprouts is that of the larval sea lamprey, *Petromyzon marinus* (Hall et al., 1989). Approximately three weeks after axotomy close to the neuronal cell bodies of the anterior bulbar cells, sprouts emerge from the dendritic tips of these cells. The dendrites proximal to these sprouts, which often appear swollen, develop some ultrastructural properties of axons, particularly presynaptic elements and abundant neurofilaments.

These structures, however, differ from normal axons in that they contain labile microtubule populations, as detected by the presence of reaction product with tyr tubulin antibodies (Hall et al., 1991). Well before the time that sprouts emerge, as early as five days after axotomy, phosphorylated neurofilaments can be detected in the dendrites (Hall et al., 1991). Under normal conditions, phosphorylated neurofilament immunoreacitivity is detectable only in axons. This finding leads to the speculation that the phosphorylation status of the neuronal cytoskeleton may be a controlling element for neurite identity. Although the association of neurofilaments with Alzheimer neurofibrillary tangles is controversial, these phosphorylated neurofilament antibodies are also capable of labeling neurofibrillary tangles.

Tau Phosphorylation and Neurodegeneration

From a cell-biology perspective, these considerations indirectly suggest the importance of phosphorylation in cells with neurofibrillary tangles. A more direct assessment of the tau phosphorylation state in tangle-rich Alzheimer brain tissue has also revealed the presence of hyperphosphorylated tau (Ksiezak-Reding et al., 1990b). This work was predicated on the identification of several protein bands from neurofibrillary tangle fractions as a modified tau protein. These bands, run by SDS-PAGE a little higher than normal tau protein, do not purify with brain microtubules, and have solubility properties distinct from normal tau. The first reference to these modified tau proteins was by the term A68 (Wolozin et al., 1986); however, these investigators did not identify the protein as tau. More recently, these proteins have been definitively identified as tau by antibody crossreactivity (Ksiezak-Reding et al., 1990a; Flament et al., 1989) and by direct protein sequencing (Lee et al., 1991). Sucrose gradient fractions containing PHF, when analyzed by gel electrophoresis, contain only these modified tau bands, clearly demonstrating that at least some PHFs are soluble in SDS (Lee et al., 1991). When the phosphate content of these proteins was analyzed, they contained 12 mol of phosphate per mole of tau, in contrast to normal brain tau from Alzheimer brain and from control brain, which contained phosphate in the range of 3 mol of phosphate/mol of tau (Ksiezak-Reding et al., 1990b). The latter phosphate contents are similar to the phosphate content of tau previously reported in bovine brain tau (Ueda et al., 1990). The distinct features of this modified tau protein are best described by the term PHF-tau. An antibody that recognizes PHF-tau, but not normal adult human tau has been described (Greenberg and Davies, 1990). Lee et al. (1991) has suggested that serine 396 is a critical site of altered phosphorylation in PHF-tau; however, the massive degree to which PHF-tau is phosphorylated compared to normal tau may imply multiple critical sites.

One functional consequence of tau hyperphosphorylation is its inability to bind to microtubules (Rosenblum et al., 1990). If tau is unable to bind to microtubules, one might predict that axonal microtubules would become destabilized. Indeed, McKee et al. (1990) have observed that an antibody to acetylated tubulin, which probes for stable microtubules, is not reactive with dystrophic neurites. However, microtubules are lost throughout tangle-bearing neurons (Ellisman et al., 1987) and dystrophic neurites often have a dendritic origin (Yamaguchi et al., 1990). Therefore, the problem of microtubule instability under the conditions of Alzheimer's disease extends beyond the axonal compartment. In fact, although PHF-tau is left behind in affected neurons, it is likely that other components of the microtubule system, including MAP2 and tubulin, have been proteolyzed, because many tangle-bearing cells lack immunoreactivity with antibodies to these proteins. Tau may be left behind in the course of massive microtubule destabilization, as an inadvertent consequence of its molecular structure.

A crucial question in Alzheimer research is that of the relationship of the neuritic lesions described here to the other classical hallmark of the disease, the deposition β-amyloid. Some causative role for the amyloid in the generation of the neuritic lesions is likely, since the β-amyloid protein appears to deposit several years before neuritic changes are apparent (Mann et al., 1988). One prominent location of dystrophic neurites is around and within the senile plaque, and direct induction of neuritic changes following the expo-sure of neurons to a putatively toxic amyloid fragment has been described (Yankner et al., 1990). However, the distribution of the β-amyloid peptide does not correlate well to the distribution of neurofibrillary tangles (Kuzuhara et al., 1989). These observations suggest that the effects of the β-amyloid peptide may be more remote, rather than directly on the neurites with which the peptide comes in contact. The formation of the amyloid fibril may result in neuronal-membrane injury that resembles axotomy to the extent that membrane integrity has been violated. Since neuritic lesions are not unique to Alzheimer's disease, other brain insults could induce similar lesions. The manifestations of the injury may vary depending on where in the very diverse structure of a neuron the injury occurs. Injury at the nerve terminus may result in a protracted time-course before neuritic changes occur; once they do occur, they may appear remote from the site of the injury. Injury closer to the cell body may have a greater impact on the integrity of the dendritic tree. It is possible that one aspect of an amyloid-related injury to neuronal cell membranes is a disruption of internal calcium homeo-stasis, a type of insult that has been associated with the induction of neuritic changes in cells in culture (Mattson, 1990). The subsequent acti-vation of calcium-dependent kinases or inhibition of phosphatases could induce much of the cyto-skeletal reorganization described here. Phos-phorylation of MAPs and their subsequent dissociation from microtubules would destabilize microtubules; concommittant neurofilament phosphorylation would, in the somatoden-dritic region, contribute to the loss of dendritic identity.

Summary

It is very likely that the neuronal ctyoskeleton plays a role in the formation and maintenence of axonal and dendritic identity. The disruption of the cytoskeleton in Alzheimer's disease involves a repartitioning of cytoskeletal elements that under normal conditions are preferentially segregated to the axon or dendrite. The setting in which this category of lesion is observed is that of neuronal injury resulting in denervation. The cytoskeleton undergoes progressive destabilization and loss of microtubules and a concommitant accumulation of highly phosphorylated filaments. In Alzheimer's disease, at least some of these filaments are likely to represent aberrantly phosphorylated tau polymers.

References

Aizawa H., Kawasaki H., Murofushi H., Kotani S., Suzuki K., and Sakai H. (1989) A common amino acid sequence in 190-kDa microtubule-associated protein and tau for the promotion of microtubule assembly. *J. Biol. Chem.* **264**, 5885–5890.

Arai H., Lee V. M., Otvos L., Jr., Greenberg B. D., Lowery D. E., Sharwa S. K., Schmidt M. L., and Trojanowski J. Q. (1990) Defined neurofilament, tau and beta-amyloid protein epitopes distinguish Alzheimer from non-Alzheimer senile plaques. *Proc. Natl. Acad. Sci. USA* **87**, 2249.

Barcikowska M., Wisniewski H. M., Bancher C., and Grundke-Iqbal I. (1989) About the presence of paired helical filaments in dystrophic neurites participating in the plaque formation. *Acta Neuropathol.* **78**, 225–231.

Binder L. I., Frankfurter A., and Rebhun L. I. (1985) The distribution of tau in the mammalian central nervous system. *J. Cell Biol.* **101**, 1371–1378.

Black M. M. and Baas P. W. (1989) The basis of polarity in neurons. *Trends Neurosci.* **12**, 211–214.

Braak H. and Braak E. (1988) Neuropil threads occur in dendrites of tangle-bearing nerve cells. *Neuropathol. Appl. Neurobiol.* **14**, 39–44.

Braak H., Braak E., Grundke-Iqbal I., and Iqbal K. (1986) Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: A third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. *Neurosci. Lett.* **65**, 351–355.

Bre M. H. and Karsenti E. (1990) Effects of brain microtubule-associated proteins on microtubule dynamics and the nucleating activity of centrosomes. *Cell Motil. Cytoskel.* **15,** 88–98.

Brion J. P., Guilleminot J., Couchie D., Flament-Durand J., and Nunez J. (1988) Both adult and juvenile tau microtubule-associated proteins are axon specific in the developing and the adult rat cerebellum. *Neuroscience* **25,** 139–146.

Caceres A. and Kosik K. S. (1990) Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature* **343,** 461–463.

Caceres A., Potrebic S., and Kosik K. S. (1991) The effect of tau antisense oligonucleotides on neurite formation of cultured cerebellar macroneurons. *J. Neurosci.* **11,** 1515–1523.

Couchie D., Charriere-Bertrand C., and Nunez J. (1988) Expression of the mRNA and tau proteins during brain development and in cultured neurons and astroglial cells. *J. Neurochem.* **50,** 1894–1899.

Crystal H., Dickson D., Fuld P., Masur D., Scott R., Mehler M., Masdeu J., Kawas C., Aronson M., and Wolfson L. (1988) Clinico-pathologic studies in dementia: Nondemented subects with pathologically confirmed Alzheimer's disease. *Neurology* **38,** 1682–1687.

Drubin D. G., Feinstein S. C., Shooter E. M., and Kirschner M. W. (1985) Nerve growth factor-induced neurite outgrowth in PC12 cells involves the coordinate induction of microtubule assembly and assembly promoting factors. *J. Cell Biol.* **101,** 1799–1807.

Drubin D., Kobayashi S., Kellogg D., and Kirschner M. (1988) Regulation of microtubule protein levels during cellular morphogenesis in nerve growth factor-treated PC12 cells. *J. Cell Biol.* **106,** 1583–1591.

Ellisman M. H., Ranganathan R., Deerinck T. J., Young S., Terry R., and Mirra S. (1987) Neuronal fibrillar cytoskeleton and endomembrane system organization in Alzheimer's disease, *Alterations in the Neuronal Cytoskeleton in Alzheimer Disease,* G. Perry, ed., Plenum, New York.

Ennulat D. J., Liem R. K. H., Hashim G. A., and Shelanski M. L. (1989) Two separate 18-amino acid domains of tau promote the polymerization of tubulin. *J. Biol. Chem.* **264,** 5327–5330.

Flament S., Delacourte A., Hemon B., and Defossez A. (1989) Characterization of two pathological tau protein variants in Alzheimer brain cortices. *J. Neurol. Sci.* **92,** 133–141.

Greenberg S. G. and Davies P. (1990) A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. *Proc. Natl. Acad. Sci. USA* **87,** 5827–5831.

Haines J. L., Ozelius L. J., McFarlane H., Menon A., Tzall S., Martiniuk F., Hirschhorn R., and Gusella J. F. (1990) A genetic linkage map of chromosome 17. *Genomics* **8,** 1–6.

Hall G. F., Lee V. M.-Y., and Kosik K. S. (1991) Microtubule destabilization and neurofilament phosphorylation precede dendritic sprouting following close axotomy of lamprey central neurons. *Proc. Natl. Acad. Sci. USA* **88,** 5016–5020.

Hall G. F., Poulos A., and Cohen M. J. (1989) Sprouts emerging from the dendrites of axotomized lamprey central neurons have axonlike ultrastructure. *J. Neurosci.* **9,** 588–599.

Himmler A. (1989) Structure of the bovine tau gene: alternatively spliced transcripts generate a protein family. *Mol. Cell. Biol.* **9,** 1389–1396.

Himmler A., Drechsel D., Kirschner M. W., and Martin D. W., Jr. (1989) Tau consists of a set of proteins with repeated C-terminal microtubule-binding domains and variable N-terminal domains. *Mol. Cell. Biol.* **9,** 1381–1388.

Horio T. and Hotani H. (1986) Visualization of the dynamic instability of individual microtubules by dark-field microscopy. *Nature* **321,** 605–607.

Ihara Y. (1988) Massive somatodendritic sprouting of cortical neurons in Alzheimer's disease. *Brain Res.* **459,** 138–144.

Kanai Y., Takemura R., Oshima T., Mori H., Ihara Y., Yanagisawa M., Masaki T., and Hirokawa N. (1989) Expression of multiple tau isoforms and micro-tubule bundle formation in fibroblasts transfected with a single tau cDNA. *J. Cell Biol.* **109,** 1173–1184.

Knops J., Kosik K. S., Lee G., Pardee J. D., Cohen-Gould L., McConlogue L. Overexpression of tau in a non-neuronal cell induces long cellular processes. *J. Cell Biol.* (in press).

Kosik K. S., Caceres A., Pardee J., Cohen-Gould L., Knops J., and McConlogue L. (1990) A role for tau in neural morphogenesis. *J. Cell Biol.* **111,** 436a.

Kosik K. S., Crandall J. E., Mufson E., and Neve R. L. (1989) Tau *in situ* hybridization in normal and Alzheimer brain: a predominant localization in the neuronal somatodendritic compartment. *Ann. Neurol.* **26,** 352–361.

Kosik K. S. and Finch E. A. (1987) MAP_2 and tau separate into axonal and dendritic domains after

the elaboration of morphologically distinct neurites: An immunocytochemical study of cultured rat cerebrum. *J. Neurosci.* **7,** 3142–3153.

Kowall N. W. and Kosik K. S. (1987) Axonal disruption and aberrant localization of tau protein characterize the neuropil pathology of Alzheimer's disease. *Ann. Neurol.* **22,** 639–643.

Ksiezak-Reding H., Binder L. I., and Yen S. H. (1990a) Alzheimer disease proteins (A68) share epitopes with tau but show distinct biochemical properties. *J. Neurosci. Res.* **25,** 420–430.

Ksiezak-Reding H., Liu W. K., Hradsky J., and Yen S. H. (1990b) Phosphate analysis of different tau preparations from normal and Alzheimer disease brains. *J. Cell Biol.* **111,** 435a.

Kuzuhara S., Ihara Y., Toyokura Y., and Shimada H. (1989) A semi-quantitative study on Alzheimer neurofibrillary tangles demonstrated immunocytochemically with anti-tau antibodies, in the brains of non-demented and demented old people. *Brain Nerve* (Tokyo) **41,** 465–470.

Lee G., Cowan N., and Kirschner M. (1988) The primary structure and heterogeneity of tau protein from mouse brain. *Science* **239,** 285–288.

Lee G., Neve R. L., and Kosik K. S. (1989) The microtubule binding domain of tau protein. *Neuron* **2,** 1615–1624.

Lee V. M. Y., Balin B. J., Otvos L., and Trojanowski J. Q. (1991) A68 proteins are major subunits of Alzheimer disease paired helical filaments and derivatized forms of normal tau. *Science* **251,** 675–678.

Lewis S. A., Ivanov I. E., Lee G.-H., and Cowan N. J. (1989) Organization of microtubules in dendrites and axons is determined by a short hydrophobic zipper in microtubule-associated proteins MAP2 and tau. *Nature* **342,** 498–505.

McIntosh J. R. and Porter M. E. (1989) Enzymes for microtubule-dependent motility (minireview). *J. Biol. Chem.* **264,** 6001.

McKee A. C., Kosik K. S., and Kowall N. W. (1990) Dendritic neurites are histochemically and morphologically distinct from axonal neurites in Alzheimer's disease. *Soc. Neurosci. Abstracts* **16,** 461.

McKee A. C., Kosik K. S., and Kowall N. W. (1991) Neuritic pathology and dementia in Alzheimer's disease *Ann. Neurol.* (in press).

McKee A. C., Kowall N. W., and Kosik K. S. (1989) Microtubular reorganization and growth response in Alzheimer's disease. *Ann. Neurol.* **26,** 652–659.

Mangin G., Couchie D., Charriere-Bertrand C., and Nunez J. (1989) Timing of expression of tau and its encoding mRNAs in the developing cerebral neocortex and cerebellum of the mouse. *J. Neurochem.* **53,** 45–50.

Mann D. M. A., Marcyniuk B., Yates P. O., Neary D., and Snowden J. S. (1988) The progression of the pathological changes of Alzheimer's disease in frontal and temporal neocortex examined both at biopsy and at autopsy. *Neuropathol. Appl. Neurobiol.* **14,** 177–195.

Mattson M. P. (1990) Antigenic changes similar to those seen in neurofibrillary tangles are elicited by glutamate and Ca^{2+} influx in cultured hippocampal neurons. *Neuron* **4,** 105–117.

Matus A., Bernhardt R., and Hugh-Jones T. (1981) High molecular weight microtubule-associated proteins are preferentially associated with dendritic microtubules in brain. *Proc. Natl. Acad. Sci. USA* **78,** 3010–3014.

Miller P., Walter V., Therkauf W. E., Vallee R. B., and DeCamilli P. (1982) Frozen tissue sections as an experimental system to reveal specific binding sites for the regulatory subunit of type II cAMP-dependent protein kinase in neurons. *Proc. Natl. Acad. Sci. USA* **79,** 5562–5566.

Mitchison T. and Kirschner M. (1984) Dynamic instability of microtubule growth. *Nature* **312,** 237–242.

Murphy D. B. and Wallis K. T. (1985) Erythrocyte microtubule assembly *in vitro*: determination of the effects of erythrocyte tau, tubulin isoforms, and tubulin oligomers on erythrocyte tubulin assembly. *J. Biol. Chem.* **260,** 12293–12301.

Neve R. L., Harris P., Kosik K. S., Kurnit D. M., and Donlon T. A. (1986) Identification of cDNA clones for the human microtubule-associated protein, tau, and chromosomal localization of the genes for tau and microtubule-associated protein 2. *Mol. Brain Res.* **1,** 271–280.

Oblinger M. M., Argasinski A., Wong J., and Kosik K. S. (1991) Tau gene expression in rat sensory neurons during development and regeneration *J. Neurosci.* (in press).

Peng A., Binder L. I., and Black M. M. (1986) Biochemical and immunological analysis of cytoskeletal domains of neurons. *J. Cell Biol.* **102,** 252–262.

Rosenblum J. S., Bramblett G. T., and Lee V. M. Y. (1990) The selective phosphorylation of sites near the microtubule (MT) binding domain in normal

tau may be an initial event leading to the formation of Alzheimer disease (AD) paired helical filaments (PHF). *J. Cell Biol.* **111,** 435a.

Sanchez I., Twersky L. H., and Cohen W. D. (1990) Detergent-based isolation of marginal bands of microtubules from nucleated erythrocytes. *Eur. J. Cell Biol.* **52,** 349–358.

Seagull R. W., Holland S., and Gibson D. M. (1990) Isolation of microtubule proteins from cotton (*Gossypium hirsutum*) suspension cells. *J. Cell Biol.* **111,** 294a.

Ueda K., Masliah E., Terry R., Saitoh T., Bakalis S. L., Scoble H., and Kosik K. S. (1990) Alz-50 recognizes a phosphorylated epitope of tau protein. *J. Neurosci.* **10,** 3295–3304.

Weingarten D., Lockwood A. H., Hwo S. Y., and Kirschner M. W. (1975) A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. USA* **72,** 1858–1862.

Wolozin B. L., Pruchnicki A., Dickson D. W., and Davies P. (1986) A neuronal antigen in the brains of Alzheimer patients. *Science* **232,** 648–650.

Yamaguchi H., Nakazato Y., Shoji M., Ihara Y., and Hirai S. (1990) Ultrastructure of the neuropil threads in the Alzheimer brain: Their dendritic origin and accumulation in the senile plaques. *Acta Neuropathol.* **80,** 368–374.

Yankner B., Duffy L., and Kirschner D. (1990) Neurotrophic and neurotoxic effects of amyloid beta-protein: Reversal by tachykinin neuropeptides. *Science* **250,** 279–282.