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Isolation of cDNAs coding for epitopes shared by microtubule-associated proteins and neurofibrillary tangles in Alzheimer's disease

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5 cross-hybridizing cDNA clones of sizes 2.2 (3 cDNAs), 1.3 and 0.8 kb corresponding to tau microtubule-associated protein have been isolated from a rat brain λgt11 expression library. Antibodies affinity-purified using the fusion protein encoded by the cDNAs were observed to label tau polypeptides on Western blots and Alzheimer's neurofibrillary tangles.

Microtubule-associated protein; Alzheimer's disease; cDNA; Cytoskeleton

1. INTRODUCTION

Several proteins promote the in vitro polymerization of tubulin and co-assemble with tubulin to form microtubules. These include tau proteins (55–68 kDa) [1], MAPs, 1 and 2 (270–350 kDa) [2], and some more recently described MAPs ranging from 120 to 240 kDa [3]. In adult rat brain, tau proteins are composed of at least 4 major polypeptides, as judged by one-dimensional SDS-polyacrylamide gel electrophoresis [1,4–6]. The polypeptide complement of tau changes as development proceeds, for example in newborn rat brain, tau has been reported to be composed of only two polypeptides which are progressively replaced by the adult pattern [7].

The different tau polypeptides have been found to be homologous by peptide mapping [8] and by immunochemical analysis, including cross-reactivity with monoclonal antibodies [4,5]. The microheterogeneity is, in part, due to different phosphorylation states [9,10] but also arises from different primary translation products [11,12].

Neurofibrillary tangles (NFT) in Alzheimer's disease are one of the neuropathological hallmarks of the disease. They comprise bundles of abnormal filaments (paired helical filaments, PHF) accumulating in neurones. PHF possess unique ultrastructural features [13], distinguishing them from microtubules and neurofilaments, the normal fibrous organelles that constitute the neuronal cytoskeleton. Despite these morphological differences, PHF share epitopes with several cytoskeletal proteins: PHF react with antibodies directed against phosphorylated epitopes present on the C-terminal domains of the largest neurofilament polypeptides (NF-H and NF-M) [14] and with antibodies against the microtubule-associated proteins tau [15–19] and MAP2 [20,21]. It is not known whether all or only some species of tau are involved in the PHF.

We report here the isolation of putative tau

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cDNAs from a rat brain expression library, and provide evidence that these cDNAs code for epitopes present on PHF.

2. MATERIALS AND METHODS

2.1. *RNA isolation and preparation of cDNA library*

Rat and post-mortem human brain RNA was prepared by the guanidinium thiocyanate extraction procedure [22]. 10 μg rat polyadenylated RNA isolated by oligo(dT)-cellulose chromatography were used to prepare cDNA [23] which was inserted into the *Eco*RI site of the bacteriophage expression vector λgt11 [24].

2.2. *Preparation of antiserum and library screening*

The rabbit polyclonal anti-tau serum [15] was stripped of anti-*E. coli* antibodies and the library screening performed as reported in [25], substituting protein A-alkaline phosphatase for protein A-peroxidase.

2.3. *Immunochemistry*

The fusion proteins encoded by each putative tau clone were transferred as λgt11 plaques from agar plates to nitrocellulose filters and antibodies were affinity-purified from the serum by elution from these nitrocellulose filters [26]. These antibodies were tested on Western blots of thermostable microtubule-associated proteins [6] prepared from microtubules polymerized in vitro [27].

The affinity-purified antibodies were also tested for their reactivity with neurofibrillary tangles by immunocytochemistry on tissue sections in light and electron microscopy [14,15].

3. RESULTS

A library of 10^6 recombinant phages was generated and 1.7×10^5 plaques screened with the anti-tau serum. Five positive clones were detected, with cDNA insert sizes of 0.8, 1.3, and three inserts of 2.2 kb, as determined by agarose gel electrophoresis. By Southern blot analysis the five

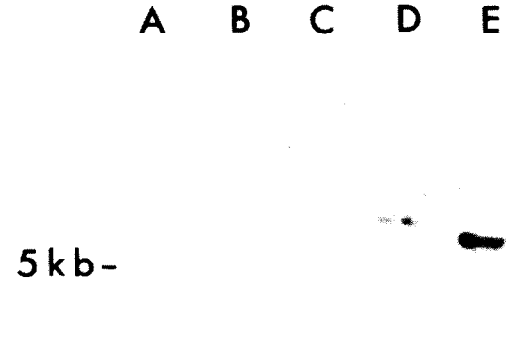

Fig.1. Northern blot analysis using ptau/6 cDNA. Lanes A–E, RNA from: A, kidney; B, muscle; C, liver; D, brain from 3 day-old rat; E, brain from adult rat.

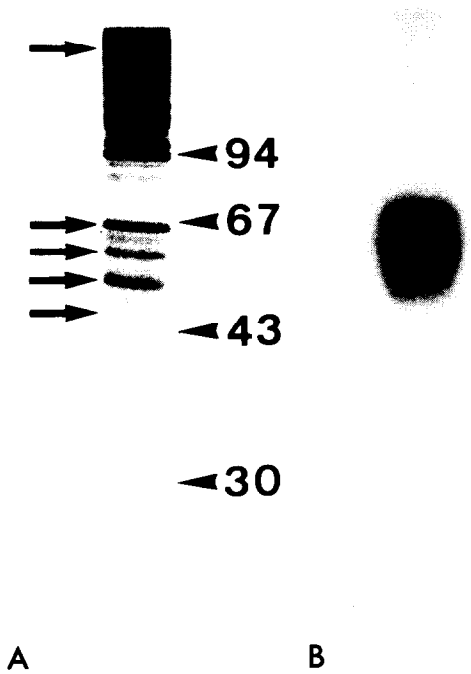

Fig.2. Characterization of the antibody selected by ptau/1 cDNA-encoded fusion protein. (A) Coomassie blue staining of rat brain thermostable microtubule-associated proteins. Arrows indicate MAP2 and tau proteins. (B) Immunoblotting of the same preparation with the antibody affinity-purified from the serum using the fusion protein expressed by ptau/1 clone.

cDNAs were observed to cross-hybridize (not shown). One of the 2.2 kb inserts, designated ptau/6, was subcloned in plasmid pAT153. Northern blot analysis of adult rat brain RNA indicated that ptau/6 cDNA gives a signal at 6 kb but no hybridization was observed on rat liver, kidney or muscle RNA (fig.1). A signal at 6.2 kb was also observed using 3-day-old rat brain RNA (fig.1D), and a weaker hybridization was seen with human RNA (not shown).

Affinity-purified antibodies labeled, in each case, several proteins with positions corresponding to 3 tau polypeptides on Western blots of thermostable microtubule-associated proteins that are enriched for tau and MAP2 (fig.2). In addition, these affinity-purified antibodies showed a weak cross-reactivity with MAP2. The fusion proteins were also labeled on Western blots by the anti-tau serum (not shown).

The same affinity-purified antibodies labeled only Alzheimer neurofibrillary tangles on tissue sections of human brain in light and electron microscopy (fig.3), indicating that the epitopes present on the putative tau fusion proteins are shared with tangles.

4. DISCUSSION

The following criteria were thus used to establish that our five clones code for putative tau mRNAs: (i) the cDNAs hybridize to a brain-specific messenger, whose size is similar to that previously reported for tau mRNA [12,28]; (ii) antibodies, affinity-purified using the fusion proteins, are specific for proteins with properties consistent with them being identified as tau polypeptides (thermostable microtubule-associated proteins with molecular mass between 50 and 70 kDa); (iii) the fusion proteins are labeled by the anti-tau serum on Western blots.

Drubin et al. [12] have reported the isolation of mouse tau cDNAs which hybridize to a 6 kb band

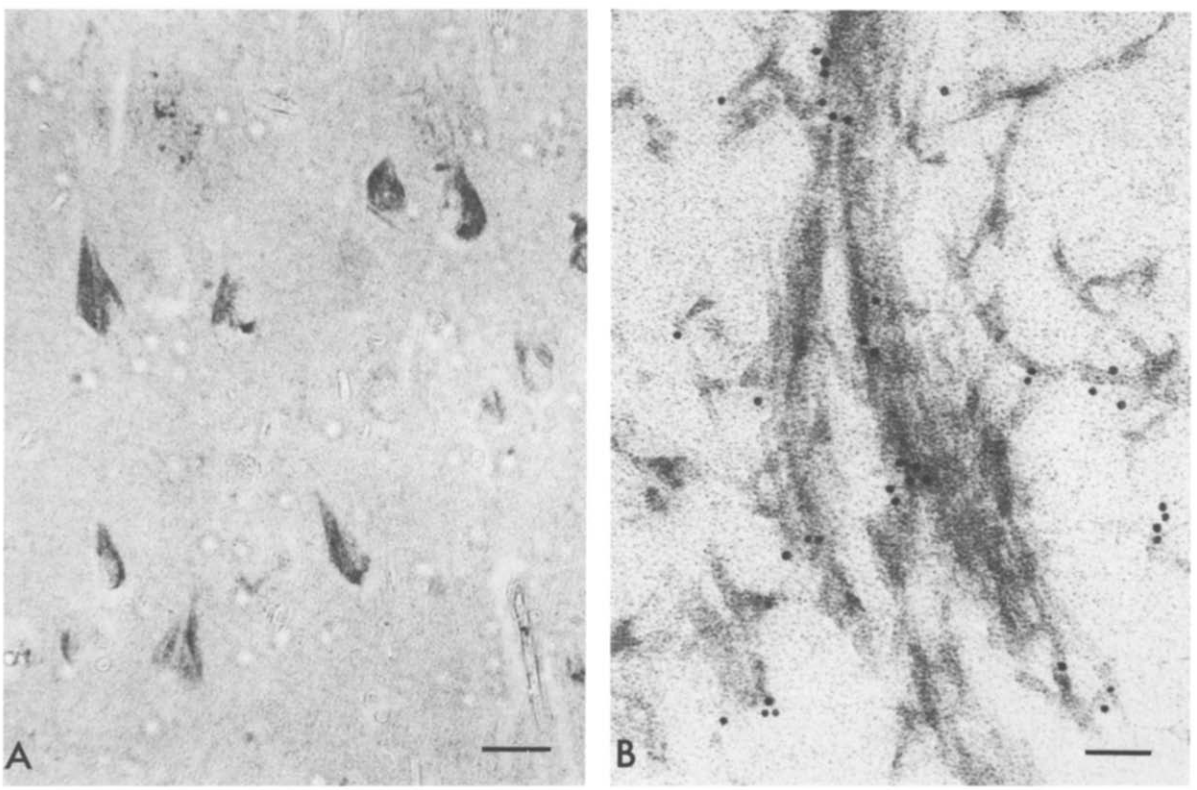

Fig.3. Immunolabeling of Alzheimer's neurofibrillary tangles by ptau/6 affinity-purified antibody. (A) Paraffin section of hippocampus; scale bar, 5 μm. (B) Ultrathin section of a cortical tissue sample; scale bar, 100 nm.

by Northern analysis. A tau mRNA of the same size in rat, mouse and fetal human brain was confirmed by Neve et al. [28], who also reported a developmental shift in the size of tau mRNA, i.e. a 6.5 kb band in adult rodent brain and a 6.0 kb in fetal and post-natal rodent brain. At variance with these reports, Ginzburg et al. [11] have observed that rat tau mRNA has a size of 2.2 kb, as judged from in vitro translation of size-fractionated RNA but the reason for the discrepancy between this report and those of Drubin et al. [12], Neve et al. [28] and our data has yet to be resolved.

Our observation that our 5 putative tau clones cross-hybridize shows that they contain sequences derived from the same messenger, or from related messengers of the same size, since only one band was detected on Northern blots. The existence of several messengers for different tau isoforms has been suggested [11,12] because tau heterogeneity is observed in in vitro translation products and according to the developmental stages from which mRNA is translated in vitro these products vary in pattern on SDS-PAGE. Definitive proof of multiple protein sequences must await sequence analysis of tau cDNAs.

The affinity-purified antibodies, even those obtained using the smallest 0.8 kbp clone, label several tau species, confirming that the latter share epitopes [4,5]. We observed that there is a developmental shift in the size of tau mRNA, although this shift is the reverse of that reported by Neve et al. [28]. This is consistent with the hypothesis of several tau mRNAs of the same range of size that show a developmental heterogeneity. Our cDNA clones could therefore be selective for different tau mRNA species from those detected by the previously published clones [28].

Affinity-purified antibodies were observed to cross-react with MAP2. A cross-reaction between MAP2 and tau has been reported [21]. However, our ptau/6 cDNA, at the stringency used, only recognized a 6 kb mRNA, whereas a 9 kb mRNA has been observed for MAP2 [29]. Tau and MAP2 can compete for the same tubulin-binding site [30] and ptau/6 could encode an immunochemically cross-reactive sequence common to the two proteins and involved in this binding site.

Axonal labeling, which has been observed by immunocytochemistry on fresh rat brain with anti-tau antibodies [4], was not observed on post-mortem human brain tissue sections and this too is a characteristic of anti-tau antibodies: tau epitopes associated with tangles are resistant to post-mortem autolysis and/or tissue processing, whereas, normal microtubule-associated tau is labile. It has also been reported that tau epitopes on neurofibrillary tangles are abnormally phosphorylated [31]. However, we show here that anti-tau antibodies against most probably unphosphorylated fusion proteins (phosphorylation being a post-translational event) label tangles. These results indicate that our putative tau cDNAs encode protein sequences that are present in tangles although the presence of fortuitously shared epitopes on unrelated proteins in tangles, rather than tau per se, cannot at this stage be excluded. Further work is now required to establish the extent of cross-reactivity between tangles and the putative tau proteins encoded by the cDNA clones and this should also serve the purpose of increasing our knowledge of the polypeptide composition of tangles.

These cDNAs may also enable us to study the genetic control of tau protein expression in normal brain and in several neurological diseases where tau protein has been found to be associated with abnormalities of the neuronal cytoskeleton.

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