Studies on the Expression of the Microtubule-associated Protein, Tau, during Mouse Brain Development, with Newly Isolated Complementary DNA Probes

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ABSTRACT  Tau protein is a collection of closely related polypeptides that associate with microtubules in vivo and stimulate their assembly in vitro. Using an affinity-purified antiserum against bovine brain tau protein, we found that the number and amount of tau polypeptides changes dramatically during mouse brain development. The different forms appear to result from changes in tau mRNA since in vitro translation products reflect the qualitative and quantitative changes found in vivo. To study the mRNA and genomic complexity of tau protein, we used tau mRNA, purified from polysomes with tau antisem, to isolate embryonic mouse tau complementary DNA clones. With these probes we have determined that embryonic tau protein is translated from a 6-kb mRNA that persists throughout brain development.

Microtubule assembly in vitro is promoted by a set of proteins called microtubule-associated proteins (MAP). These proteins coassemble with tubulin into microtubules, and have been located on microtubules in vivo (8, 9). Two major classes of MAP have been distinguished, the lower molecular weight tau proteins of 55,000-62,000 mol wt and the higher molecular weight MAP of ~300,000 mol wt (5, 19, 23, 30, 32). In addition, MAP of intermediate molecular weight have been described in primate cells (2). Within each class of proteins there is considerable heterogeneity.

Tau protein was originally found to be quite heterogenous, appearing as four or five major bands in mature pig brain when examined on one-dimensional SDS-polyacrylamide gels and five times that number on two-dimensional gels (5). Recently a 50,000-mol-wt coated vesicle protein (26) as well as neuroblastoma MAP of 69,000 and 80,000 mol wt (10), have been shown to be related to brain tau proteins. Despite this heterogeneity, the pig brain tau species that are resolved on one-dimensional gels are virtually indistinguishable when compared by peptide mapping and amino acid analysis (5, 6).

The close relatedness of the different tau polypeptides suggests they share a function, such as binding to microtubules. The existence of many different forms of tau protein on the other hand has raised questions as to the biological significance of the heterogeneity. Does this heterogeneity represent functional differences in the proteins, strategies for cellular localization, developmental regulation, or even steps in degradation? These are important questions, but the extreme heterogeneity of tau protein itself complicates considerably the study of its structure and activity.

To begin our examination of the function of tau protein and to find a role for its heterogeneity, we studied changes in tau protein during mouse brain development, and the level at which this heterogeneity is generated. As a further step in understanding the structural basis of this heterogeneity, we have isolated complementary DNA (cDNA) probes for an embryonic form of tau protein in mouse brain. Using these probes, we have begun to study the relationship among the various forms of tau protein, and how tau protein expression is regulated.

MATERIALS AND METHODS

Analysis of Brain Tau Proteins:  Brain extracts were prepared immediately after killing ICR mice, or several hours postmortem for beef brain, by homogenizing tissue in ice-cold polymerizing buffer (0.1 M morpholinoethanesulfonic acid, 0.5 mM MgCl₂, 2 mM EGTA, 0.1 mM EDTA, 1 mM β-mercaptoethanol, pH 6.4) supplemented with 0.5 mM PMSF, 1 mM aminooacetoniitrile, and 10 mM benzamidine HCl, in a motor-driven Teflon pestle. The homogenate was centrifuged for 30 min, 4°C at 40,000 rpm in a Beckman 50Ti rotor (Beckman Instruments, Spinco Div., Palo Alto, CA). An aliquot was assayed for protein (21) and the remainder was boiled in SDS sample buffer and stored at ~20°C. Microtubule protein was purified by the Winograd et

Abbreviations used in this paper:  cDNA, complementary DNA; kb, kilobase; MAP, microtubule-associated proteins; SSC, 150 mM NaCl, 15 mM Na citrate.
al. modification (32) of the Shelanski et al. procedure (29), and tubulin by chromatography on phosphocellulose (32). Tau protein was purified by the method of Herzog and Weber (17).

SDS PAGE was carried out according to Laemmli (20) and immunoblotting experiments, by the procedure of Burnette (3). Preparation of the affinity-purified bovine tau antiseraum was described previously (26). Immune complexes were separated by the procedure of Burnette (3). Preparation of the affinity-dextran-1-mm gels slices were placed in an equal volume of 1% 0-mercaptoethanol (ME) in a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). After gentle shaking for 15 min at room temperature, particulate material was pelleted for 5 min at 10,000 rpm in a Sorvall (DuPont Instruments-Sorvall Biomedical Div., Wilmington, DE) centrifuge. The homogenate was incubated overnight at 4°C and precipitated RNA was collected by centrifugation at 4,000 rpm for 15 min. The pellet was washed twice in homogenization buffer, resuspended in 1 ml of 10 mM Tris-Cl (pH 7.4), 10 mM NaCl, then extracted once with an equal volume of phenol. Poly-A* RNA was isolated from total RNA by one cycle of chromatography on oligo(dT)-cellulose. Poly*A* RNA recovery was assayed by the ability of the poly A to hybridize to [3H]polyU and thus protect the [3H]-polyU from a single-strand specific nuclease. An ~100-fold molar excess of [3H]polyU over poly A was incubated with polyA* RNA in 2x SSC (1X SSC = 150 mM NaCl, 15 mM Na citrate) at 45°C for 15 min, then on ice for 5 min. RNase A was added to 10 ug/ml for 15 min on ice. 100 ng of carrier DNA was then added, trichloroacetic acid was added at 5%, and the precipitating containing proteins [3H]polyU was collected on a Whatman GF/C filter. The filter was dried and scintillation-counted in Aquasol (New England Nuclear, Boston, MA). To fractionate mRNA by molecular weight, 10 µg in 40 mM methymercuric hydroxide was run at 50 V for 5 h on a low-melting-agarose (Sea Plaque, Marine Colloids, Rockland, ME) gel containing 5 mM methymercuric hydroxide. 1-mm gel slices were placed in an equal volume of 1% 0-mercaptoethanol, 0.2% SDS, 10 mM Tris-CI (pH 7.4), 1 mM EDTA, 0.1 M NaCl, and boiled for 20 s. Samples were immediately vortexed for 1 s, phenol extracted until interface material disappeared, and ethanol precipitated twice. mRNA was translated in a rabbit reticulocyte lysate system (Bethesda Research Laboratories, Gaithersburg, MD) containing [35S]methionine (25). Translation products were either directly analyzed by SDS PAGE followed by fluorography with E/M/HANCE (New England Nuclear) or were first immunoprecipitated for tau.

To immunoprecipitate tau proteins, we boiled 30 µl of translation reaction with 4.5 µl and 10% SDS and added to 105 µl of lysis buffer (25 mM Tris-CI [pH 7.4], 0.4 M NaCl, 0.1% deoxycholate, 0.1% Nonidet P-40). This mixture was used to resuspend 30 µl of washed and pelleted Staphylococcus aureus cells (Panisorbin; Calbiochem-Behring Corp., San Diego, CA). After 10 min at room temperature, the samples were centrifuged and the preloaded supernatants were transferred to tubes containing 55 µl phosphate-buffered saline and 125 µl affinity-purified tau antiseraum or an equivalent amount of nonimmune rabbit IgG (Cappel Laboratories). After 20 min at room temperature, 30 µl of a 10% suspension of S. aureus cells, washed once in phosphate-buffered saline and resuspended in phosphate-buffered saline supplemented with 10 µg per milliliter of bovine serum albumin and 1 mg per milliliter of methionine, was added. After 5 min at room temperature, the cells were collected by centrifugation. The cells were washed twice in 200 µl lysis buffer with 0.5% SDS and once in 200 µl 10 mM Tris-CI (pH 7.4), 5 mM EDTA, each time collecting cells by centrifugation through a 0.6-ml cushion of 0.25 M NaCl, 25 mM sucrose, 0.5% deoxycholate, 0.1% Nonidet P-40, 0.2% Triton X-100, 0.5% sodium deoxycholate, 5 mM EDTA, and 1 M sucrose. The pellets were resuspended in 10 µl of SDS sample buffer, boiled for 3 min, centrifuged and 5 µl of the supernatant was added to an RIPAbuffer (25). The supernatant was boiled for 3 min, centrifuged and 5 µl of the supernatant was added to an RIPAbuffer (25).

Electrophoresis and Blotting of DNA and RNA: DNA was isolated from total RNA by one cycle of chromatography on oligo(dT)-cellulose. Poly*A* RNA recovery was assayed by the ability of the poly A to hybridize to [3H]polyU and thus protect the [3H]-polyU from a single-strand specific nuclease. An ~100-fold molar excess of [3H]polyU over poly A was incubated with polyA* RNA in 2x SSC (1X SSC = 150 mM NaCl, 15 mM Na citrate) at 45°C for 15 min, then on ice for 5 min. RNase A was added to 10 ug/ml for 15 min on ice. 100 ng of carrier DNA was then added, trichloroacetic acid was added at 5%, and the precipitating containing proteins [3H]polyU was collected on a Whatman GF/C filter. The filter was dried and scintillation-counted in Aquasol (New England Nuclear, Boston, MA).

RESULTS

Analysis of Cow and Mouse Brain Tau Proteins

Affinity-purified antiserum against bovine brain tau protein was used on nitrocellulose transfers to analyze the forms of tau protein present in cow and mouse brain extracts. Fig. 1, a-c, shows tau polypeptides from cow brain. In lane a the soluble brain extract was probed with antibody to tau. Six major bands with molecular weights between 47,000 and 61,000 were observed. The tau antigens in microtubule protein and in purified tau protein are shown in lanes b and c of Fig. 1. The lowest band in the total brain extract, lane a, disappears as tau protein is purified by coassembly into microtubules (Fig. 1, lane b). An additional major low molecular weight antigen is lost in the further purification of tau (Fig. 1, lane c). Low molecular weight tau polypeptides were also detected when tau protein was purified directly from brain without coassembly with tubulin (4).

Major changes in tau antigens occur during mouse brain development. Between 12 and 15 d of fetal development (Fig. 1, d and e), tau polypeptides of 47,000–50,000 mol wt (a doublet in lighter exposures) increase in abundance relative to total protein, while between 5 and 15 d after birth (Fig. 1,
FIGURE 1 Immunoblot analysis of cow (lanes a–c) and mouse (lanes d–j) tau proteins. Tau polypeptides in total bovine brain extract (lane a), bovine microtubule protein (lane b), and thermal-stable, phosphocellulose-purified bovine tau protein (lane c) were compared. Lanes d–h contain 20 μg of 12-d-old fetus, 15-d-old fetus, 5-d-old newborn, 15-d-old newborn, and adult brain proteins, respectively. Lanes i and j contain 20 μg of adult mouse cerebrum and cerebellum proteins, respectively. (Minor low molecular weight species here and in Fig. 2 may be proteolytic products from tau since they appear below, but not above, the tau proteins.)

Characterization of the Heterogeneity of Tau by In Vitro Translation of Tau mRNA and the Purification of tau mRNA

To study the heterogeneity of tau protein at the mRNA level, tau protein was immunoprecipitated from in vitro translation reactions using RNA from various sources (Fig. 2). The four tau polypeptides resulting from the in vitro translation of cow brain mRNA (Fig. 2, lane 1) resemble those seen in highly purified cow tau protein (Fig. 1, lane c). The patterns of tau polypeptides synthesized with 6-d-mouse mRNA (Fig. 2, lane 5) and adult mouse mRNA (Fig. 2, lane 3) again resemble those seen in total brain extracts (Fig. 1, lanes f and h). They are, however, not identical, suggesting that the protein products may be modified or have differential stability.
in adult brain of that size. Both the translation of 6-d mouse brain mRNA and the immunoblot of proteins from the same developmental stage show two major species at a molecular weight of 47,000-50,000. In the adult brain the translation reaction shows decreased amounts of a tau species at 47,000-50,000 mol wt. Translation of 6-d and adult mRNA both show proteins of 62,000 mol wt, although no 62,000-mol-wt protein was detected in early mouse extracts (Fig. 1, lane f). Adult mRNA directs the synthesis of additional faint translation products of 50,000-62,000 mol wt, which may correspond to peptides in adult brain of that size.

To study the complexity of tau on the RNA level, it was necessary to isolate a tau cDNA clone. We chose to use 6-d old mouse brain as a source of mRNA, since the immunoblotting experiments (Fig. 1) and the translation experiments (Fig. 2) showed that tau protein and tau mRNA levels are highest at that stage. First, two characteristics of tau protein, its ability to efficiently coassemble into microtubules (4) and its stability to heat treatment (32) were used to further demonstrate that the immunoreactive translation products from 6-d mouse brain mRNA were tau. Before immunoprecipitation the translation products are seen as a heterogeneous array of proteins, but two to four closely spaced bands are seen after immunoprecipitation with anti-tau serum (Fig. 2, lane 7). When the entire translation mixture was added to tubulin, which was polymerized into microtubules, the major translation products that pelleted were a- and b-tubulin (Fig. 2, lane 8). When the microtubule pellet was boiled, the supernatant (Fig. 2, lane 9) was highly enriched in tau proteins (compare lanes 7 and 9). This experiment reinforces the conclusion that the major translation products recognized by tau antisera have the properties of tau proteins.

To enrich for tau mRNA to make cDNA clones, we reacted polysomes isolated from 6-day mouse brain with tau antisera and the polysomes with nascent tau peptides were isolated by passage over a protein A–Sepharose column.

The extent of purification of tau mRNA can be estimated by quantifying the proportion of protein immunoprecipitated from translation products directed by total mouse brain mRNA or by immunopurified tau mRNA. Separate experiments showed that the immunoprecipitation is 30% efficient. Of the 864,000 cpm incorporated using total brain mRNA, the tau antibody precipitates only 390 cpm, shown in Fig. 3, lane 2, indicating that tau mRNA represents <0.15% of the total. Preimmune serum precipitates nothing (Fig. 3, lane 1). In the absence of added mRNA the reticulocyte lysates incorporate no counts that can be immunoprecipitated by tau antisera (Fig. 3, lane 4). However, out of only 29,000 cpm derived from translation of immunopurified tau mRNA, 900 cpm (10.2% of total mRNA) can be immunoprecipitated by tau antisera (Fig. 3, lane 6) indicating a 60-fold purification. We therefore expected 10% of the clones derived from this mRNA to contain tau sequences.

**Isolation and Characterization of Tau cDNA Clones**

Bacterial clones containing tau cDNA were identified as those that hybridized to radiolabeled probes made from immunopurified tau mRNA, but not from total 6-d mouse brain mRNA. In close agreement with the percentage of positive clones we expected (see above), 11% of the colonies (81 out of 737) were positive. These colonies contained cDNA of two types that did not cross-hybridize when examined by blot analysis (data not shown). A large representative of each type (pTA1 and pTA2), with inserts of 1,300 and 1,900 base pairs was used in each of the experiments described below.

To confirm that plasmids pTA1 and pTA2 contain sequences homologous to tau mRNA, they were fixed to nitrocellulose filters and used to select tau mRNA out of total 6-d mouse brain mRNA. The amount of tau mRNA bound to each filter was assayed by translating the mRNA and immunoprecipitating the resulting translation products with tau antisera. We included control filters in the experiment that did not contain putative tau clones. Since under our incubation conditions each filter nonspecifically binds some of the total brain mRNA, we expected to see a faint tau protein immunoprecipitate from the control filters. However, we expected that if a filter contained a tau cDNA clone, the immunoprecipitated tau signal should be increased over background. Fig. 4, lane b, shows the immunoprecipitated signal from 250,000 cpm of the translation products directed by total brain mRNA, while lanes c-f in Fig. 4 show signals from only 60,000 cpm of translation products directed by RNA selected by cloned DNA. Fig. 4, lane c, shows the background signal from mRNA selected by a control filter containing a b-tubulin cDNA clone, while Fig. 4, lane f, shows another control with a blank nitrocellulose filter. The signal in lane b of Fig. 4 is about four times as intense as that in lanes e and f, which is expected since four times as many cpm were used in lane b as were used in lanes c and f. When plasmids pTA1 and pTA2 were tested, each gave strong tau signals when compared with controls (Fig. 4, compare lanes d and e to lanes c and f). Note that the signals in lanes d and e are more intense than the signal in lane b, despite the fact that four
FIGURE 4 Identification of tau cDNA by hybrid-selected translation. Lanes a and b are immunoprecipitates from 250,000 cpm of translation products directed by total 6-day brain mRNA using nonimmune serum (a) or tau antiserum (b). Lanes c–f are immunoprecipitates using tau antiserum on 60,000 cpm of translation products directed by mRNA bound to filters containing β-tubulin cDNA (lane c), pTA2 (lane d), pTA1 (lane e), or no DNA (lane f).

Complexity of Tau mRNA and Genes

One possible explanation for the failure of pTA1 and pTA2 to cross-hybridize is that they represent different parts of a large mRNA. To investigate this possibility, we probed mRNA and genomic DNA blots with pTA1 and pTA2. Fig. 5 shows pTA2 hybridizing to 5-, 10-, 15-, 20-day, and adult mouse brain mRNA. A single band of 6-kilobase pair (kb) appears in every sample and there is little change in relative abundance. The hybridization pattern is identical when pTA1 is used instead of pTA2 (not shown, but for related data see Fig. 7).

To confirm that the tau translational activity resides in a 6-kb mRNA, we fractionated 6-d mouse brain mRNA on a denaturing agarose gel, eluted from slices, translated, and the resulting products were immunoprecipitated. Fig. 6 shows that all of the tau translational activity resides in the 5.3-7 kb range, with a peak at 6 kb. No appreciable tau translational activity is found at lower molecular weight. To answer the question of whether the two tau clones represent different genes whose mRNA products cannot be resolved by gel electrophoresis or represent different regions of the same gene, mouse genomic DNA digested with different restriction enzymes was separated by electrophoresis, blotted, and probed with either pTA1 (Fig. 7A, lanes 1–3), or pTA2 (Fig. 7A, lanes 4–6). Both probes hybridize to the same major band in Bam HI, Hind III, and EcoRI digests. However, pTA2 hybridizes faintly to several additional bands not hybridized by pTA1.

As a further test of the relationship between pTA1 and pTA2, we tested for related genomic sequences in species other than mouse, as suggested by the conserved nature of tau protein (6). As shown in Fig. 7B, pTA1 and pTA2 hybridize to the same fragments in mouse, human, and chicken genomic DNA. However, pTA2 hybridizes to human DNA more strongly than pTA1, and, in addition, hybridizes to frog, and perhaps Drosophila DNA, suggesting it may contain more highly conserved sequences than pTA1.

DISCUSSION

Tau protein from hog brain was originally described as four or five closely related polypeptides of 55,000–62,000 mol wt that facilitate the in vitro assembly of tubulin into microtubules. Since the purification of tau protein involved successive warm and cold incubations of crude brain extract, it was possible that tau heterogeneity arose adventitiously in vitro. We examined tau polypeptides directly in brain extracts made in the presence of protease inhibitors and boiled in SDS (Fig. 1). Not only does tau heterogeneity exist in crude cow and mouse brain extracts, but the heterogeneity is developmentally programmed in mouse. Developmentally regulated changes in tau protein may be a general feature of mammalian
standards at 5 and 2 kb).

This region. (Mouse ribosomal RNAs were used as molecular weight
and 0-tubulin and actin which are heavily translated by mRNAs in
and below in the immunoprecipitations are background due to s-

a 1% agarose gel containing methylmercury hydroxide. The 7.6-
antiserum (PI) or tau antiserum (I). Total mRNA was fractionated on
6-d mouse brain mRNA. Translation products directed by total
mRNA were subjected to immunoprecipitation using nonimmune
antisera. Two criteria were used to first identify these clones. First,
they were positive when screened with a probe made
from immunoselected tau mRNA, but not when screened
with probe made from unselected total brain mRNA. Second,
the clones hybridize to tau mRNA as judged by hybrid-
selected translation experiments. That two non-overlapping
sets of clones were isolated may be the result of an internal
priming site for reverse transcriptase in the cDNA reaction.
Perhaps the mRNA has two stable configurations that expose
different priming sites. Alternatively, the S1 nuclease after second cDNA strand synthesis (15) may have cut the
tau cDNAs in half if an AT rich region was present in the
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To study the nature of tau heterogeneity on a genetic level,
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The embryonic tau cDNA clones were used to study the
source of tau heterogeneity on the genetic level. We were
surprised to find that embryonic tau protein is made from a
6-kb mRNA since only 1.3 kb of coding sequence is required
for a 50,000-mol-wt protein. Even more surprising is the fact
that only a single mRNA was detected throughout brain
development, despite the fact that two tau species of 47,000-
50,000-mol-wt occur in early mouse brain and more species
arise later. How then, can we account for the heterogeneity
of tau protein? One possibility is that our probe recognizes
multiple mRNAs that are unresolved on our gels. The
mRNAs could be the products of different genes or could result from developmentally regulated differential mRNA
processing of a common precursor mRNA. Differential
mRNA splicing accounts for different forms of several proteins
(1, 11). Another possibility is that different tau mRNAs exist for different tau polypeptides that are resolved on our
gels, but that our probes are specific for only one of those
mRNAs. We have cloned 3.2 kb of a 6-kb mRNA. If our
clones did not contain coding sequence, only the single ho-

mologous mRNA would be recognized. We think that our
probes do contain coding sequences, since they are phyloge-
netically conserved. Noncoding sequences tend not to be
conserved (7), although there are exceptions. If multiple

How does tau protein heterogeneity arise? One possibility
is that all tau polypeptides are cleaved from a common
precursor protein. This is not likely since much of the cow
and mouse tau protein heterogeneity observed in brain
extracts (Fig. 1) can be generated by in vitro translation of
corresponding mRNAs (Fig. 2). This is particularly clear for the
translation of cow brain mRNA which, as shown in
Fig. 2, lane I, gives four major species corresponding to the
upper four bands in Fig. 1, lanes a–c. In mouse, changes in
the 47,000–50,000-mol-wt tau polypeptides from 5-d old to
adult mouse (Fig. 1, lanes f and h) are reflected by translating
the corresponding mRNAs (Fig. 2, lanes 5 and 3). However,
the 62,000-mol-wt tau polypeptide present in adult, but not
in 5-d mouse, appears to be synthesized by both mRNAs.
This may reflect a higher turnover of the 62,000-mol-wt
mouse tau early in brain development, or the masking of its
mRNA until later in development. The lowest two cow tau

species seen in Fig. 1, lanes a and b, however, are either not
primary translation products, or are not antigenic when trans-
lated. It has been reported that all rat tau species can also be
generated by in vitro translation and that some rat tau
mRNAs appear in the brain before their corresponding pro-
teins accumulate (14). These results suggest that different
mRNAs exist for different tau species.

We studied embryonic mouse tau protein. Fig. 1, lanes d–j,
shows that embryonic tau, which occurs as a doublet of
47,000–50,000 mol wt, peaks in abundance around the time
of birth and diminishes through development, being largely
replaced by higher molecular weight tau species. When em-
byronic tau is synthesized in in vitro translation reactions,
two to four proteins of 47,000–50,000 mol wt are observed
(Fig. 2, lane 7). Characteristic of tau proteins, these proteins
efficiently coassemble with tubulin into microtubules and are
thermal stable. It is interesting that the primary translation
products should efficiently coassemble into microtubules,
since neuroblastoma and pheochromocytoma tau proteins are
preferentially phosphorylated when associated with microtu-
bules (24). If phosphorylation is necessary for coassembly, the
primary translation products must be phosphorylated in the
reticulocyte lysate or by the added tubulin before assembly.
Further anlayis is necessary to see whether this occurs.

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mRNAs do exist through development, we may be able to detect them by doing RNA blots at lower hybridization stringencies.

When we cut the 6-kb 6-d mRNA out of a gel and translated it (Fig. 6), we observed the same degree of heterogeneity seen in early mouse brain (Fig. 1, lane f), where a 47,000-50,000 mol wt tau doublet occurs. (What appears to be a single species >47,000 mol wt in Fig. 6 is actually a doublet, seen on close inspection.) This doublet may be generated by two mRNAs that co-migrate at 6 kb. Alternatively, the doublet could be made from a single mRNA. How could two polypeptides arise from a single mRNA? If our cell-free translation system is faithfully translating tau protein, the heterogeneity may be due to correct in vivo posttranslational modifications (which also occur in the reticulocyte lysate). Alternatively, there may be built-in imprecision in the translational initiation and/or termination signals on the tau mRNA. Finally, although we know of no precedent for a nonviral polycistronic mRNA in a eucaryote, we note that the tau mRNA is large enough to code multiple polypeptides of 50,000 mol wt.

In the case of cow tau protein, we have preliminary evidence suggesting that there are multiple 6-kb tau messages (Drubin and Kirschner, manuscript in preparation). On Northern blots of cow mRNA probed with mouse tau cDNA probes, we see a single band co-migrating with the mouse tau mRNA at 6 kb. When we fractionated cow polyA+ RNA and translated it as was done for mouse mRNA in Fig. 6, all of the four tau bands seen in Fig. 2 (lane 1) were translated from two of the fractions, containing mRNA of ~7-5 kb. However, the translation products resulting from the fraction containing larger mRNA were enriched in the uppermost tau band. This result suggests that separate mRNAs exist for different cow tau species. In addition, this result demonstrates that our mouse tau cDNA probe is able to hybridize to cow tau mRNA. In summary, we have shown that tau heterogeneity is developmentally programmed and is not principally due to proteolysis during purification; the possibilities for generating this heterogenety are extensive, but the tau cDNA clones and high titer antisera against the protein will allow us to answer these questions.

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