

Brain-specific Expression of MAP2 Detected Using a Cloned cDNA Probe

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Abstract. We describe the isolation of a set of overlapping cDNAs encoding mouse microtubule associated protein 2 (MAP2), using an anti-MAP antiserum to screen a mouse brain cDNA expression library cloned in bacteriophage λ gt11. The authenticity of these clones was established by the following criteria: (a) three non-identical clones each expressing a MAP2 immunoreactive fusion protein were independently isolated from the expression library; each of these clones cross-hybridized at the nucleic acid level; (b) anti-MAP antiserum was affinity purified using nitrocellulose-bound fusion protein; these antibodies detected only MAP2 in an immunoblot experiment of whole brain microtubule protein; (c) a series of cDNA "walking" experiments was done so as to obtain a non-overlapping cloned fragment corresponding to a different part of the same mRNA molecule. Upon subcloning this non-overlapping fragment into plasmid expression vectors, a fusion protein was synthesized that was immunoreactive with an anti-MAP2 specific antiserum. Thus, a single contiguous cloned mRNA molecule encodes at least two MAP2-specific epitopes; (d) the cloned cDNA probes detect an mRNA species in mouse brain that is of a size (~9 kb)

consistent with the coding capacity required by a 250,000-D protein.

The MAP2-specific cloned cDNA probes were used in RNA blot transfer experiments to assay for the presence of MAP2 mRNA in a variety of mouse tissues. Though brain contained abundant quantities of MAP2 mRNA, no corresponding sequences were detectable in RNA prepared from liver, kidney, spleen, stomach, or thymus. We conclude that the expression of MAP2 is brain-specific.

Use of the MAP2 specific cDNA probes in genomic Southern blot transfer experiments showed the presence of a single gene encoding MAP2 in mouse. The microheterogeneity of MAP2 is therefore ascribable either to alternative splicing within a single gene, or to posttranslational modification(s), or both. Under conditions of low stringency, the mouse MAP2 cDNA probe cross-hybridizes with genomic sequences from rat, human, and (weakly) chicken, but not with sequences in frog, *Drosophila*, or sea urchin DNA. Thus, there is significant interspecies divergence of MAP2 sequences. The implications of the above observations are discussed in relationship to the potential biological function of MAP2.

TEMPERATURE-DEPENDENT polymerization of microtubules *in vitro* results in the co-assembly, together with α - and β -tubulin, of a group of proteins known collectively as the microtubule associated proteins (MAPs).¹ These proteins promote the microtubule assembly process *in vitro* (8, 18, 19, 24, 25, 32) and at least some of them have been localized as projections from the surface of the microtubule polymer (1, 11, 14). Thus, while the precise biological function of MAP remains to be clearly demonstrated, they could potentially mediate the interaction of microtubules with other cellular elements as well as serve in the control of microtubule polymerization.

Two major groups of MAP have been defined on the basis

1. *Abbreviations used in this paper:* MAP, microtubule associated protein; SSC, 150 mM NaCl, 15 mM Na citrate; TBST, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20.

of molecular weight: the high molecular weight MAP (in the range 240,000–350,000 D) and the low molecular weight tau proteins (55,000–62,000 D) (8). The high molecular weight group contains two members, MAP1 (>300,000 D) and MAP2 (250,000–300,000 D) (24), that are biochemically and immunologically distinct. Each group exhibits heterogeneity on one-dimensional SDS polyacrylamide gels: in the case of MAP1, three discrete but closely migrating bands, named MAP1a, 1b, and 1c, have been identified, whereas MAP2 can be resolved into two components termed MAP2a and 2b (30).

The molecular basis for the heterogeneity of the high molecular weight MAP is unclear. While protein chemical characterization (for example, analysis of proteolytic digestion products) has been useful in showing the relatedness of some MAP (e.g., MAP2a and 2b [10, 21]), such experiments cannot distinguish between the expression of two similar but non-

identical genes, and the expression of one gene to yield a single protein that could then undergo posttranslational modification(s). To investigate the heterogeneity of the high molecular weight MAPs at the genetic level, we decided to construct MAP-specific cDNA clones that could be used as probes to assay the number of genes encoding each MAP group. Such clones will also serve as a basis for understanding the structure, expression, evolution, and function of these proteins. Here we report the isolation of cDNA clones encoding mouse MAP2. These clones have been used to show the existence of a single mouse gene encoding the MAP2 polypeptide, with expression restricted exclusively to brain tissue.

Materials and Methods

Construction and Screening of a Mouse Brain cDNA Library

Mouse brain polyA⁺ mRNA was prepared from 8-d-old mice by the guanidine isothiocyanate procedure (3). Double-stranded cDNA was synthesized from this material and inserted into the bacteriophage λ gt11 vector (35) as described (16). Approximately 5×10^5 recombinant plaques were screened using a rabbit anti-rat MAP antiserum (23) at a dilution of 1:500. Clones giving a positive signal with the avidin/biotin/peroxidase complex procedure (Vector Laboratories, Burlingame, CA) were picked, purified to homogeneity, and the cDNA inserts subcloned into plasmid pUC8 for further amplification and study.

Identification of Overlapping Clones by cDNA "Walking"

Excised inserts from clones that express immunoreactive fusion proteins, or restriction fragments derived from such clones, were ³²P-labeled by nick translation and used to screen the cDNA library (2) for cross-hybridizing sequences. After hybridization, the filters were washed to a final stringency of $2 \times$ SSC (150 mM NaCl, 15 mM Na citrate), 68°C. Clones giving positive signals were picked as 5-mm plugs using the open end of a Pasteur pipet, and the bacteriophage eluted in 1 ml of 0.1 M NaCl, 10 mM Tris-HCl, pH 8.0, 10 mM MgSO₄. An aliquot (30 μ l) of the eluted bacteriophage was amplified in a small (1 ml) culture without further purification, and DNA prepared from each lysate by the method described (17). The final product was dissolved in 40 μ l of 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 20 μ l of this solution digested with *Eco*RI under the conditions specified by the supplier (New England Biolabs, Beverly, MA). The digestion products were resolved on a 1% agarose gel, the gel content transferred to nitrocellulose (27), and the blot hybridized with $\sim 10^7$ cpm of the same probe(s) used to screen the cDNA library (see above). After washing to $2 \times$ SSC, 68°C, the blots were exposed to film. This procedure served to identify those plugs that contained bacteriophage with large recombinant inserts homologous to the probes used to screen the library. Eluates from plugs identified in this manner were subjected to two rounds of plaque purification; the recombinant insert from a single isolated plaque giving a positive hybridization signal with the labeled probe was then subcloned into pUC8 and amplified for further study by restriction mapping and analysis in RNA blot transfer experiments.

Production of Anti-Mouse MAP1 and MAP2 Antisera and Immunoblotting

Two-cycle purified microtubules were prepared from the brains of adult mice (22). For the preparation of MAP1, the high molecular weight MAP were resolved on preparative SDS gels as described (34), the MAP1 polypeptides excised and used directly as immunogens in rabbits (9). For the preparation of MAP2, a heat stable fraction that contained primarily MAP2 was prepared as described (11), further purified on an SDS polyacrylamide gel, and used directly as immunogen (9). Sera were tested for their specificity on immunoblots (28) prepared from one-cycle mouse brain microtubules: immunoreactive bands were detected with the avidin/biotin/peroxidase reagents used according to the procedures recommended by the supplier (Vector Laboratories).

Preparation of Sera Purified by Affinity to a Fusion Protein Expressed in Bacteriophage λ gt11

Approximately 6×10^4 bacteriophage of either wild-type λ gt11 or λ gt11 expressing clone 56 (see Fig. 4) were plated out and induced for 2 h at 37°C by

overlying with a nitrocellulose filter saturated with 10 mM isopropyl β -D-thiogalactopyranoside (35). The filters were removed, rinsed in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20 (TBST), and blocked for 30 min at room temperature in TBST that contained 3% bovine serum albumin (BSA). Each filter was then immersed for 1 h in 0.1 ml of affinity purified anti-rat MAP antibody (23) diluted 1:100 in TBST that contained 3% BSA. After three 5-min rinses in TBST, the bound antibody was eluted for 15 min at room temperature with 3 ml of 100 mM glycine-HCl, pH 2.6, 150 mM NaCl. The nitrocellulose was removed, the solution adjusted to pH 7.5 with 1 M Tris, and stabilized by the addition of 2 ml of TBST that contained 3% BSA.

RNA Preparation and DNA and RNA Blot Transfer Experiments

RNA for blot transfer analysis on 0.8% gels that contained formaldehyde (6) was prepared by the guanidine isothiocyanate procedure (3). Genomic DNAs from mouse, rat, human, chicken, frog, *Drosophila*, and sea urchin were digested with restriction enzymes under the conditions specified by the manufacturer (New England Biolabs). Restriction digests of DNA were resolved on 0.8% agarose gels and transferred to nitrocellulose as described (27).

Results

Isolation of Clones from a cDNA Expression Library Using a MAP-specific Antiserum

To isolate cDNA clones encoding MAP, we constructed a cDNA expression library in λ gt11 using polyA⁺ mRNA from the brains of 8-d-old mice. Approximately 5×10^5 clones were screened for the expression of MAP using an affinity-purified polyclonal rabbit anti-rat MAP-specific antibody (23). Seven clones that yielded a positive signal with the anti-MAP antiserum were obtained. Each positive plaque was

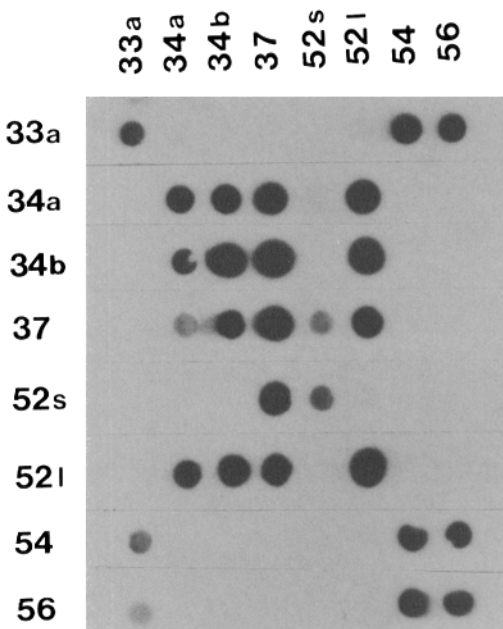


Figure 1. Two noncross-hybridizing sets of overlapping cDNA clones expressing anti-MAP immunoreactive fusion proteins. Screening of a mouse brain cDNA expression library resulted in the isolation of seven clones each yielding a positive signal with a MAP-specific antiserum. The recombinant inserts were excised, ³²P-labeled by nick translation (20), and tested in a dot-blot experiment to determine the sequence relationships among them. In the figure, 52l and 52s refer to the large and small *Eco*RI fragments contained in a single recombinant (see Fig. 4).

purified to homogeneity through two successive cycles of host infection, in each case expressing fusion proteins that reproducibly reacted with the antibody. After amplification and subcloning into plasmid vectors, the recombinant inserts were isolated and tested for their ability to cross-hybridize with one another (Fig. 1). The data show that the seven clones fall into two distinct, non-cross-hybridizing classes. This result was not unexpected, since the antibody used to screen the cDNA expression library recognizes both MAP1 and MAP2 (see below) and, in any event, the potential existed for recognizing more than one epitope on non-overlapping cloned fragments from the same mRNA.

While the generation of positive signals by reaction of an antibody with fusion proteins expressed in λ gt11 provides some indication of success, further experimental evidence is required to establish that the cloned fragments do indeed encode the desired product. This is because a large cDNA expression library cloned without regard to orientation or frame could lead to the synthesis of fusion proteins that contained epitopes adventitiously recognized by the antiserum used to screen the library (16). In addition, it is not inconceivable that the antibody might recognize a cross-reacting non-MAP protein present at very low abundance, but nonetheless represented in the cDNA library. Conventionally, the authenticity of cloned cDNAs is established by hybrid selection of mRNA and translation of the selected material in a cell-free system (e.g., 15, 16). The translation product can then be characterized either biochemically or

immunochemically or both. However, attempts to characterize our cDNA clones in this manner yielded multiple translation products immunoprecipitable with the anti-MAP antiserum, a result that we ascribe to the synthesis of an array of prematurely terminated polypeptides in the cell-free system (data not shown). Such premature termination might be expected to occur given the large size of MAP and the limitations on protein synthesis *in vitro*. We therefore did the following kinds of experiment to characterize our clones. (a) Antibodies selected by their affinity for the immunoreactive fusion protein encoded by a cDNA clone were prepared and used to probe blots of total mouse brain microtubule protein, in order to determine with which MAP (if any) the fusion protein shared common epitopes. (b) Fusion proteins were generated from non-overlapping regions of cDNA molecules cloned from a single mRNA species and tested for their ability to react independently with anti-MAP antisera. Three antisera were used for these experiments: the affinity purified anti-MAP antiserum used to screen the library (23), as well as an anti-mouse MAP1 antiserum and an anti-mouse MAP2 antiserum raised especially for this purpose. The specificity of these sera was determined by the immunoblotting experiments depicted in Fig. 2.

Characterization of the Antibody Selected by cDNA-encoded Fusion Proteins

To determine the specificity of the antibodies that recognized the immunoreactive fusion proteins, an experiment was done

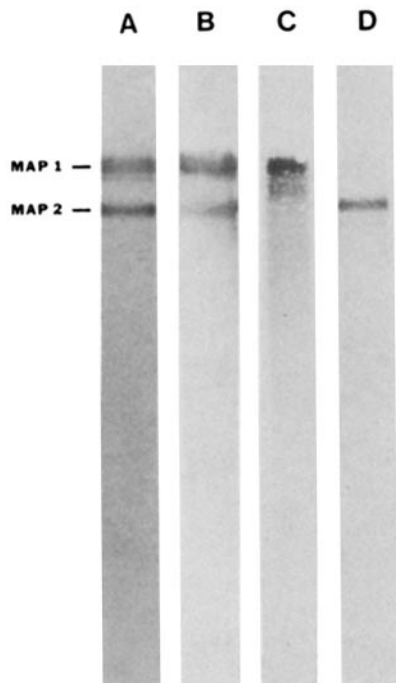


Figure 2. Characterization of MAP1- and MAP2-specific antisera. The rabbit anti-rat MAP antibody (23) used to screen the λ gt11 cDNA expression library and two additional rabbit antisera raised against purified mouse MAP1 and mouse MAP2 were characterized in an immunoblotting experiment using one-cycle purified mouse brain microtubules resolved on a 5% SDS polyacrylamide gel that contained 25% glycerol (34). (A) Coomassie Blue stain; (B, C, and D) immunoblots stained and the anti-rat MAP antibody, anti-mouse MAP1 antibody, and anti-mouse MAP2 antibody, respectively.

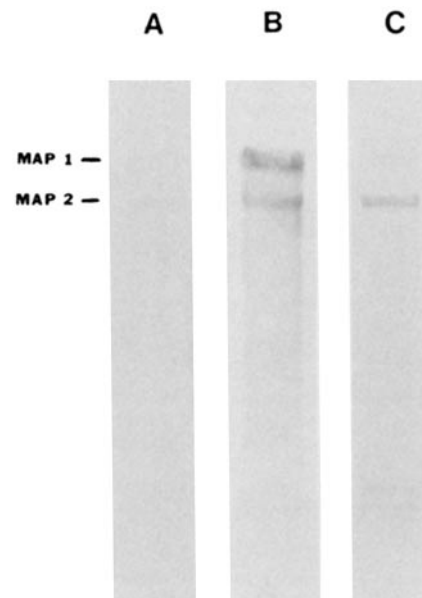


Figure 3. A fusion protein immunoreactive with an anti-MAP antibody selectively binds antibodies that detect MAP2, but not MAP1. The fusion protein expressed by clone 56 (Figs. 1 and 4) was bound to a nitrocellulose filter and used to select antibodies from the anti-rat MAP antiserum used to screen the cDNA expression library. Antibodies binding to the filter were eluted and used in an immunoblotting experiment with one-cycle purified mouse brain microtubule protein. (A) Negative control (filter-bound proteins from a non-recombinant λ gt11 clone); (B) positive control (using antibody remaining after filter selection); (C) antibody bound and eluted from a filter that contained the fusion protein expressed by clone 56 (see Fig. 4).

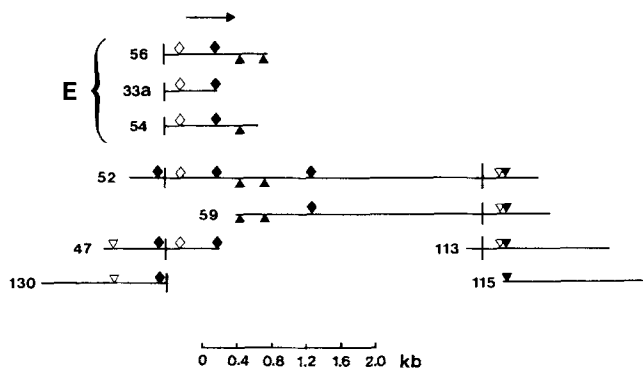


Figure 4. Restriction maps of overlapping cDNA clones obtained by cDNA "walking" experiments. A series of "walking" experiments was done in which the cDNA clones expressing MAP2-specific immunoreactive fusion proteins (marked *E* in the figure) and a number of successively overlapping clones were used to rescreen the λ gt11 cDNA library. The extent of overlap (as determined by the location of common restriction sites) is shown in the figure. The transcriptional orientation, as determined by restriction mapping of the original expression clones in λ gt11 with respect to the lac Z promoter, is indicated by the arrow. Vertical bars denote *EcoRI* sites. The translation termination codon, determined from the size of the fusion protein expressed by clone 59 (see text and Fig. 5), lies downstream from the internal *EcoRI* site common to clones 52, 59, and 113. \diamond , *KpnI*; \blacklozenge , *PstI*; ∇ , *SacI*; \triangledown , *BglI*; \blacktriangle , *HindIII*.

in which the fusion proteins were fixed to a nitrocellulose filter and allowed to react with the antiserum used to screen the cDNA expression library. Bound antibody was then eluted from the filter and its specificity determined in an immunoblotting experiment using total mouse brain microtubule protein resolved in a 5% SDS polyacrylamide gel that contained 25% glycerol (10). The result (Fig. 3C) showed that

antibody selected by its affinity for the fusion protein from one of the two sets of cross-hybridizing clones (clones 33a, 54, and 56, Fig. 1) exclusively recognized MAP2. Thus, the fusion proteins within this group contained one or more epitopes in common with authentic MAP2.

A Single Contiguous Cloned cDNA Encodes at Least Two MAP2-specific Epitopes

Because an epitope can span a region as small as about five or six amino acids, a single MAP2-specific epitope might conceivably be generated by the adventitious expression of a cDNA molecule resulting from the cloning of an unrelated mRNA in the wrong orientation and/or reading frame (see, e.g., reference 16). On the other hand, the probability of a single cDNA molecule encoding two or more adventitiously expressed MAP2-specific epitopes is vanishingly small. To obtain a series of cloned cDNAs spanning an extended region of a single, contiguous mRNA molecule, a series of cDNA "walking" experiments was done in which the initial recombinant fragments isolated from the expression library were used to rescreen the library for overlapping molecules that served to further extend the cDNA. In each case, the authenticity of the overlapping clones was determined by two criteria: (a) the isolation of multiple overlapping clones sharing common restriction sites; and (b) the detection of an identical mRNA species by each overlapping clone when used as a labeled probe in an RNA blot transfer experiment with total mouse brain RNA (see below). The results of these "walking" experiments yielded a series of overlapping cDNAs representing a contiguous stretch of ~7 kb; the extent of each cloned cDNA and its restriction map are shown in Fig. 4. Inspection of the figure shows that, as a result of "walking," a cDNA clone (clone 59) was obtained that is non-overlapping with

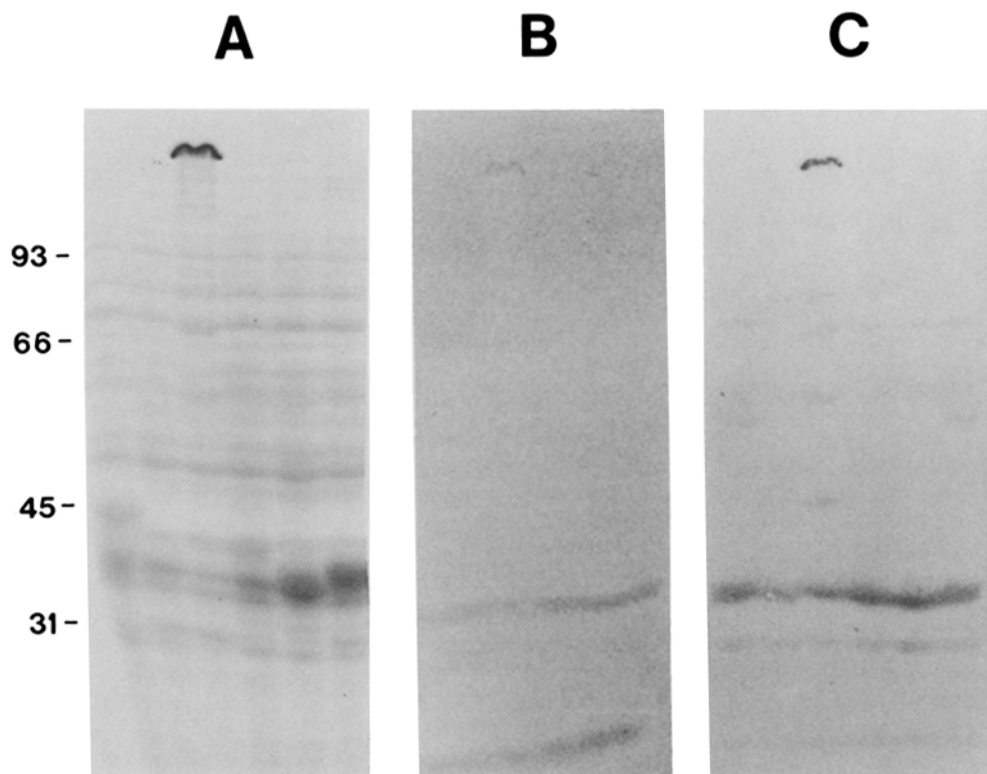


Figure 5. Two non-overlapping cDNAs encoding a contiguous mRNA molecule express MAP2-specific immunoreactive fusion proteins. The large *EcoRI* fragment from clone 59 (Fig. 4) which does not overlap clone 33a (one of the original expression clones in λ gt11) was subcloned in both orientations and all three reading frames into inducible plasmid expression vectors (see text). The resulting fusion proteins were analyzed by staining with (A) Coomassie Blue, and immunoblotting with (B) the anti-rat MAP antiserum used to screen the cDNA expression library (23) and (C) the anti-mouse MAP2-specific antibody. In each track of A, the unique prominent band is the *trpE* fusion protein. Each lane represents a different reading frame for the cloned insert.

respect to one of the original clones (clone *33a*) isolated from the cDNA expression library. The large *EcoRI* fragment from clone 59 was then inserted into inducible plasmid expression vectors (generously provided by T. J. Koerner, Duke University, NC) in all six reading frames, and the resulting fusion proteins tested for their immunoreactivity with either the anti-rat-MAP antiserum (23) or the MAP2-specific antiserum described above (see Fig. 2). In one orientation and reading frame, the larger *EcoRI* fragment from clone 59 expressed a 150,000-D fusion protein that was detected by our anti-MAP antiserum (Fig. 5) of which 37,000 D are accounted for by the *trpE*-encoded portion. In addition, and as an added control, the MAP1-specific antiserum did not recognize this fusion protein (see accompanying paper, Fig. 3.II.3). Thus, two non-overlapping fragments (*33a* and the large *EcoRI* fragment of 59, Fig. 4) cloned from a single contiguous mRNA molecule both encode separate MAP2-specific epitopes.

Size and Tissue-specific Expression of MAP2 mRNA

Though MAP2 is abundantly expressed in neuronal cells, the expression of this protein in non-neuronal tissue, determined in immunocytochemical studies using fluorescent polyclonal or monoclonal antisera (4, 7, 9, 12, 13, 29, 33, 34), is a matter

of controversy. We therefore did RNA blot transfer experiments using our MAP2-specific cloned probes to detect MAP2-specific mRNA in brain and a variety of other mouse tissues. This experiment also served to establish the size of MAP2-specific mRNA. The data (Fig. 6) reveals a single molecular species of ~9 kb that is present in brain, but is undetectable in other mouse tissues.

A Single Gene Encodes MAP2 in Mouse

In view of the existence of two resolvable MAP2 subcomponents (MAP2a and MAP2b), an important question is whether these polypeptides are encoded by separate though related genes, or whether a single gene encodes one (or more) MAP2 polypeptide(s) which can then undergo posttranslational modification. To determine the number of genomic sequences homologous to our MAP2-specific cloned cDNA probes, a genomic Southern blot transfer experiment was done. Because the mRNA encoding MAP2 is large (~9 kb, Fig. 6), we selected several probes for this experiment, three of which lacked recognition sites for one or more of the restriction enzymes used to digest the genomic DNA. The blots were hybridized and washed at relatively low stringency so as to allow the detection of related genes that might share

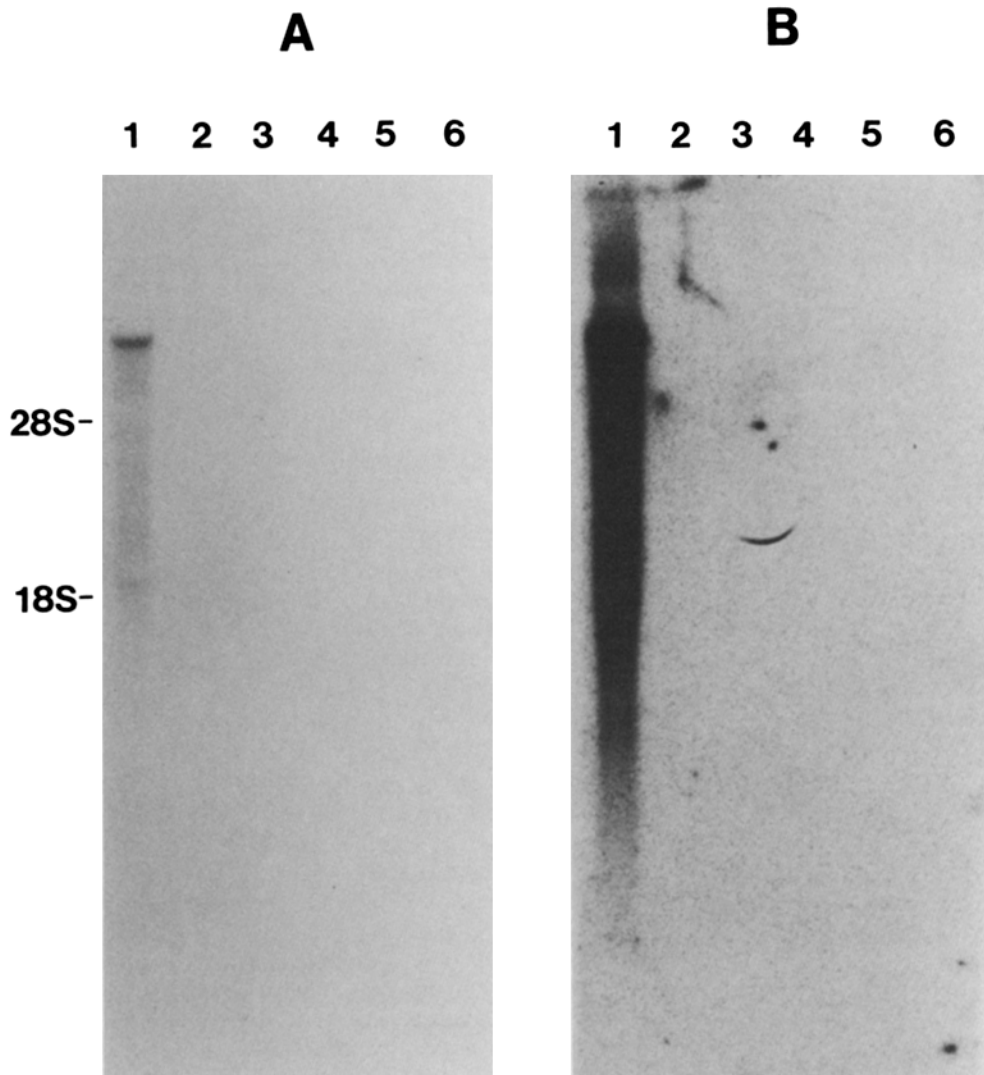


Figure 6. A large (~9-kb) mRNA encoding MAP2 is expressed exclusively in mouse brain. Aliquots (15 μ g) of polyA⁺ RNA prepared from mouse tissues were resolved in an 0.8% agarose gel that contained 1.5 M formaldehyde (6) and the gel content transferred to nitrocellulose (27). The blot was prehybridized and hybridized with the excised insert from clone 56 (Fig. 4) ³²P-labeled by nick-translation (20). Lanes 1–6, RNA from brain, kidney, liver, spleen, stomach, and thymus, respectively. (A) 12-h exposure; (B) same blot as A exposed for 500 h.

some homology with a portion of the cloned MAP2 cDNA. As seen in Fig. 7, even at this stringency these three fragments (59 [large *EcoRI* fragment], 56, 59 [small *EcoRI* fragment]) detect only one *Bam*H1 fragment and one *Kpn*I fragment (or two in the case of 56, which is cut once by *Kpn*I). However, clone 47 detects four fragments in each digest. Because these signals remain even at very high stringency (70°C, 0.2 × SSC, data not shown), they most likely reflect the interruption of the MAP2 gene by large introns that occur in the region encoded by clone 47, rather than a very close homology between a different gene and this very small portion of the MAP2 mRNA.

Limited Interspecies Conservation of the MAP2 Gene

The existence of MAP2 has been reported in cells from a number of different species (26, 29, 31). However, apart from the ability of MAP2-specific antisera to detect MAP2 across species boundaries, the extent of evolutionary conservation of the gene encoding MAP2 is unknown. To investigate this question, a Southern blot experiment was done using our labeled cloned cDNAs to probe digests of genomic DNA from rat, human, chicken, frog, sea urchin, and *Drosophila*. While

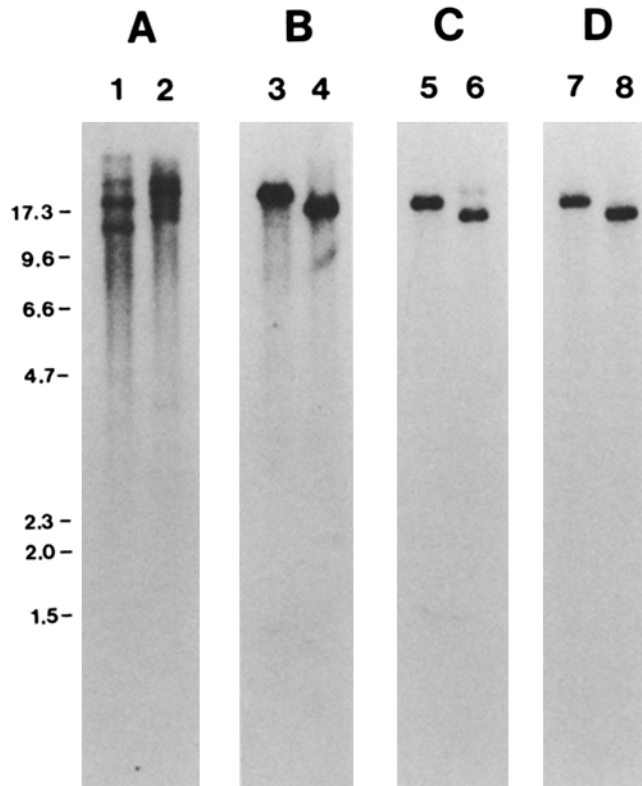


Figure 7. A single copy gene encodes mouse MAP2. The two excised *EcoRI* fragments from clone 59, and the excised inserts from clones 47 and 56 (Fig. 4) were each ³²P-labeled by nick-translation (20) and used to probe genomic Southern blots (27) of restriction digested mouse DNA. After hybridization, the blots were washed to a final stringency of 2× SSC, 42°C, so as to allow the detection of any homologous but mismatched sequences (see text). Lanes 1, 3, 5, and 7, *Bam*HI; lanes 2, 4, 6, and 8, *Kpn*I. The blots shown in A, B, C, and D were probed with labeled inserts from clones 47, 59 (large *EcoRI* fragment), 56, and 59 (small *EcoRI* fragment), respectively. Size markers (in kb) are shown at left.

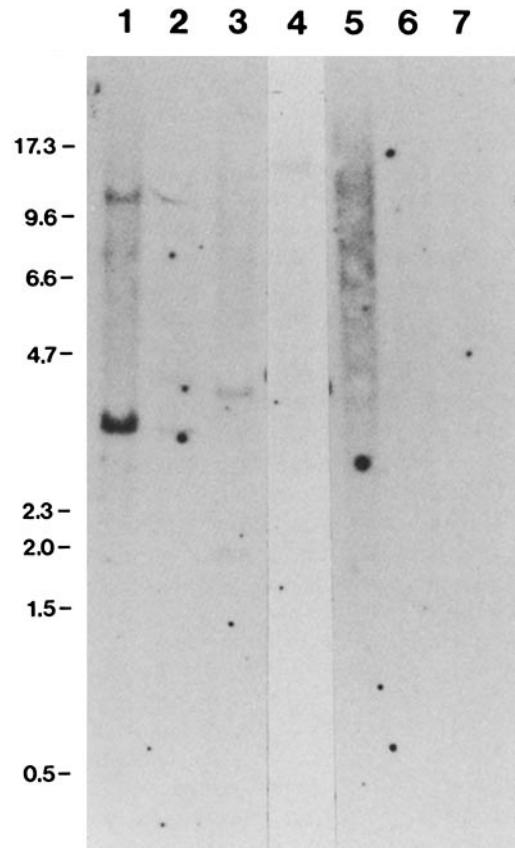


Figure 8. The gene encoding mouse MAP2 cross-hybridizes with rat, human, and chicken DNA, but not with DNA from frog, *Drosophila*, or sea urchin. The two excised *EcoRI* fragments from clone 59 and the excised insert from clone 56 were ³²P-labeled by nick-translation (20), mixed, and used to probe a Southern blot (27) prepared using *Hind*III digests of 5 μg of genomic DNA. After hybridization, the blot was washed as described in Fig. 7. Lanes 1–7, DNA from mouse, rat, human, chicken, frog, sea urchin, and *Drosophila*. Size markers (in kb) are shown at the left.

there is cross-hybridization at low stringency (2× SSC, 42°C) with DNA from rat, human, and (weakly) chicken DNA, no discrete bands were obtained with DNA from frog, sea urchin, or *Drosophila* (Fig. 8). The background hybridization detected at low stringency in frog DNA was not retained on washing to higher stringencies (data not shown). We conclude, therefore, that there is limited interspecies conservation among the sequences encoding MAP2.

Discussion

This paper describes the isolation of a series of overlapping cDNA clones encoding mouse MAP2. The isolation procedure depended on screening a mouse brain cDNA library cloned in bacteriophage λgt11, using a polyclonal rabbit anti-rat MAP antiserum (23) that recognizes both MAP1 and MAP2. While the independent isolation of two sets of cross-hybridizing cDNAs (Fig. 1) each yielding an immunoreactive fusion protein was indicative of the successful identification of MAP-specific clones, several further experiments were done to establish their authenticity. First, the fusion protein expressed by the largest member of one set of clones selected

antibodies from the antiserum used to screen the expression library that specifically recognized MAP2. Second, cDNA "walking" experiments were done so as to generate a non-overlapping cloned fragment (with respect to one of the original clones) that expressed a fusion protein recognized by both an affinity purified anti-MAP antiserum and an antiserum specific for mouse MAP2 (Fig. 5). Third, the cloned cDNA probes detected an mRNA species in RNA blot transfer experiments of a size consistent with the coding requirement of a protein of 250,000 D. On the basis of these data, we conclude that the set of overlapping cDNAs shown in Fig. 4 are specific for MAP2.

The expression of MAP2, as determined by immunocytochemical methods, is a matter of controversy. A number of reports describe the detection of MAP2 polypeptides, using defined antibodies to MAP2, in non-neuronal cells or tissues (26, 31, 34). In contrast, studies by Bernhardt, Huber, and Matus (5) using monoclonal antisera, showed the expression of MAP2 exclusively in association with dendritic microtubules in the adult brain. Our data, in which the mouse MAP2-specific cDNA probe was used to detect MAP2-specific mRNA sequences in a variety of mouse tissues (Fig. 5), is consistent with this conclusion, as we failed to detect even trace quantities of MAP2-specific mRNA from any source other than brain. The sensitivity of the hybridization procedure used in these experiments is such that we would have detected MAP2-specific mRNA sequences at a level 10^3 times lower than that present in brain. It appears likely, therefore, that the observed immunostaining of non-neuronal cells with anti-MAP2 antisera is due to the existence of cross-reacting epitopes on non-MAP2 proteins.

Genomic Southern blots probed with three different and largely non-overlapping MAP2-specific cDNA clones (see Fig. 4) identified single bands in digests of mouse DNA, even under conditions of relatively low stringency ($2 \times$ SSC, 42°C) (Fig. 7). The possibility that MAP2a and MAP2b could be transcribed from two genes that do not cross-hybridize at this stringency is highly unlikely: the two proteins give very similar or identical patterns in one-dimensional (21), two-dimensional (10), and phosphorylation (21) peptide mapping experiments, and one would expect the extent of protein homology implied by these data to be reflected at the DNA level. For example, the genes encoding different members of the relatively distantly related intermediate filament multigene family cross-hybridize at this stringency (16). In addition, over two-thirds of the MAP2 coding region was used in our cross-hybridization studies. Thus, we conclude that MAP2 is encoded by a single gene in mouse. The resolution of MAP2 into two subcomponents is therefore ascribable either to splicing of alternative exons from within a single gene, or to posttranslational modification of MAP2 protein.

Under reduced stringency conditions, the MAP2 specific cDNA clones detect one or two cross-hybridizing restriction fragments in digests of rat, human, and chicken DNA, but not in DNA from frog, *Drosophila*, or sea urchin (Fig. 8). Thus, within the region of MAP2 represented by the series of clones shown in Fig. 2, there is considerable interspecies divergence. It is conceivable that the biological function of MAP2 is consistent with a significant amount of neutral drift, so that the same function is provided by molecules encoded by weakly or non-cross-hybridizing genes. Alternatively, the

observed lack of interspecies conservation may reflect selective constraints on small, discrete regions of the molecule or regions not encoded by the set of MAP2-specific clones described here.

Because microtubules are a feature of virtually all eukaryotic cells, the absence of detectable MAP2 expression in non-neuronal tissues implies that this protein is certainly not essential to the process of microtubule assembly. Furthermore, because of the absence of related sequences with detectable homology to the MAP2-specific clones, the existence of closely related molecules that might serve a similar function to MAP2 in non-neuronal cells appears unlikely. It seems probable, therefore, that the biological role of MAP2 is brain-specific. The availability of cloned probes encoding MAP2 should provide the opportunity for fresh approaches towards studying the regulation of MAP2 gene expression as well as defining the precise biological function of this protein.

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