

# A Cloned cDNA Encoding MAP1 Detects a Single Copy Gene in Mouse and a Brain-abundant RNA Whose Level Decreases during Development

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**Abstract.** Screening of a bacteriophage  $\lambda$ gt11 cDNA expression library with a polyclonal anti-microtubule associated protein (MAP) antiserum resulted in the isolation of two non-cross-hybridizing sets of cDNA clones. One set was shown to encode MAP2 (Lewis, S. A., A. Villasante, P. Sherline, and N. J. Cowan, 1986, *J. Cell Biol.*, 102:2098–2105). To determine the specificity of the second set, three non-overlapping fragments cloned from the same mRNA molecule via a series of “walking” experiments were separately subcloned into inducible plasmid expression vectors in the appropriate orientation and reading frame. Upon induction and analysis by immunoblotting, two of the fusion proteins synthesized were shown to be immunoreactive with an anti-MAP1-specific antibody, but not with an anti-MAP2-specific antibody. Since these MAP1-specific epitopes are encoded in non-overlapping cDNAs cloned from a single contiguous mRNA, these clones cannot encode polypeptides that contain adventitiously cross-reactive epitopes. Furthermore, these cDNA clones detected an abundant mRNA species of >10 kb in mouse brain, consistent with the coding requirement of a 350,000-D polypeptide and

the known abundance of MAP1 in that tissue.

The MAP1-specific cDNA probes were used in blot transfer experiments with RNA prepared from brain, liver, kidney, stomach, spleen, and thymus. While detectable quantities of MAP1-specific mRNA were observed in these tissues, the level of MAP1 expression was ~500-fold lower than in brain. The levels of both MAP1-specific and MAP2-specific mRNAs decline in the postnatal developing brain; the level of MAP1-specific mRNA also increases slightly in rat PC12 cells upon exposure to nerve growth factor. These surprising results contrast sharply with reported dramatic developmental increases in the amount of MAP1 in brain and in nerve growth factor-induced PC12 cells.

The cDNA clones encoding MAP1 detect a single copy sequence in mouse DNA, even under conditions of low stringency that would allow the detection of related but mismatched sequences. The cDNAs cross-hybridize with genomic sequences in rat, human, and chicken DNA, but not with DNA from frog, *Drosophila*, or sea urchin. These data are discussed in terms of the evolution and possible biological role of MAP1.

**T**HE principal components of all microtubules are the  $\alpha$ - and  $\beta$ -tubulins. When these proteins are purified by co-polymerization into microtubules in vitro, a number of additional polypeptides, defined as the microtubule associated proteins (MAPs),<sup>1</sup> consistently co-purify through multiple cycles of polymerization and depolymerization (9, 29). Two groups of high molecular weight MAP, MAP1 and MAP2, are evident in microtubule preparations from mammalian brain, both of which stimulate microtubule polymerization in vitro (18, 24, 29). Immunocytochemical studies have localized these proteins on the surface of the microtubule polymer, where they appear in the electron microscope as

protuberant structures that could potentially act as cross-linking fibers in the cytoskeletal matrix (2, 7, 11, 12, 18, 21, 24, 28, 31, 32). However, the role of MAP in vivo remains uncertain.

Both MAP1 and MAP2 can be resolved into closely migrating bands of similar molecular weight on low percentage SDS polyacrylamide gels (6, 17). MAP2 consists of two sub-components, MAP2a and MAP2b, both transcribed from a single gene that is expressed exclusively in brain (23). MAP1, on the other hand, typically consists of three resolvable polypeptides, MAP1a, MAP1b, and MAP1c. The relationship between these latter molecules is unclear. Whereas peptide mapping data suggests some relatedness in structure (17), there seem to be some significant immunological and biochemical differences (3, 6), and the genetic basis of MAP1

1. *Abbreviations used in this paper:* MAP, microtubule associated protein; NGF, nerve growth factor; SSC, 150 mM NaCl, 15 mM Na citrate.

heterogeneity is unknown. Moreover, the tissue distribution of MAP1 polypeptides *in vivo* is a matter of controversy, with conflicting reports of these proteins either in a wide variety of non-neuronal cell types (7, 33), or exclusively in neurons of the adult brain, where they are predominantly present in dendrites (5, 19, 20).

The isolation of cloned cDNAs encoding the high molecular weight MAP would serve to define the genetic basis of MAP heterogeneity, as well as provide approaches to defining their structure and function. Such cloned probes should also allow the determination of patterns of cell- and tissue-specific expression based on the detection of mRNA sequences (rather than epitopes), and can be used to define evolutionary relationships among MAP polypeptides in different species. In the accompanying paper, we describe the isolation of cDNA clones encoding MAP2, and show that the expression of a single MAP2 gene is restricted exclusively to brain. Here we describe the isolation of a set of overlapping cloned cDNAs encoding MAP1. These probes detect a single gene, with preponderant (though not exclusive) expression in brain. The probes have also been used to monitor the expression of MAP1 mRNA in PC12 cells, where there is a reported dramatic increase in the synthesis of MAP1 protein in response to induction of differentiation by nerve growth factor (NGF) (13, 14, 16). However, the data reveal only a minor elevation in the abundance of MAP1-specific mRNA under these conditions.

## Materials and Methods

Construction and screening of a mouse brain cDNA expression library, extension of clones by "walking," DNA and RNA blot transfer procedures, and the generation and characterization of mouse anti-MAP1 and anti-MAP2-specific antisera are described in the accompanying paper (23). RNA was prepared by the guanidine isothiocyanate procedure (4) from mouse tissues or from rat PC12 cells (15) (generously provided by Lloyd Greene) exposed for varying times to NGF.

## Results

### Stratagem for the Identification of cDNA Clones Encoding MAP1 from a $\lambda$ gt11 Expression Library Using a MAP-specific Antiserum

The construction and screening of a  $\lambda$ gt11 cDNA expression library using a MAP-specific rabbit anti-rat MAP antiserum (28) is described in the accompanying paper (23). This antiserum was chosen because it detects both MAP1 and MAP2, and therefore offered the potential of simultaneously detecting fusion proteins in the  $\lambda$  library that contained epitopes for either MAP1 or MAP2. This approach proved successful, in that two sets of cross-hybridizing clones were obtained, with no detectable cross-hybridization between each set. Upon further analysis, one set of clones proved to encode MAP2 (23). A number of experiments were done to establish the identity of the second set of overlapping clones. As in the case of MAP2, attempts to establish the nature of the protein encoded by these clones by hybrid selection (using brain mRNA) and translation of selected material in a cell-free system yielded a spectrum of immunoprecipitable translation products of <200,000 D that could not be characterized further (data not shown). Furthermore, while the set of  $\lambda$ gt11

cDNA clones encoding MAP2 fusion proteins gave a conspicuously positive signal upon assay with the anti-MAP antiserum, the signal from the second set of cDNA clones was considerably weaker relative to the background (Fig. 1). The significant background signal could not be the result of contaminating anti-*E. coli* antibodies in the anti-MAP antiserum, since the latter was affinity purified (28); rather, it appears likely that MAP proteins contain one or more epitopes in common with *E. coli* antigens. This interpretation is consistent with our observation that absorption of the affinity-purified anti-MAP antiserum against a crude *E. coli* lysate resulted in a reduction in intensity of both signal and background (data not shown). Therefore, for the clones expressing weakly immunoreactive fusion proteins, it was not possible to use the fusion proteins expressed by the originally isolated clones to select antibodies for the determination of their specificity by immunoblotting. As an alternative, we used anti-MAP1 and anti-MAP2 specific antisera (23) as a means to distinguish between clones encoding these two proteins.

### At Least Two MAP1-specific Epitopes Are Encoded by Non-overlapping Cloned cDNAs

Fragments derived from the ends of the cloned cDNAs detected in the  $\lambda$ gt11 expression library as expressing weak but nevertheless positively immunoreactive fusion proteins (Fig. 1B) were used as labeled DNA probes to rescreen the library so as to obtain extended overlapping cloned fragments. To exclude the possibility that such overlapping fragments might contain cloning artifacts, multiple isolates were examined by restriction mapping for the presence of common restriction sites within the region of overlap. Restriction maps of the set of overlapping clones obtained in this manner are shown in Fig. 2. Three cloned *EcoRI* fragments (clones A, B, and D, Fig. 2) were each inserted in both orientations into three plasmid vectors (Koerner, T. J., Duke University, personal communication; see also [23]) designed to express cloned

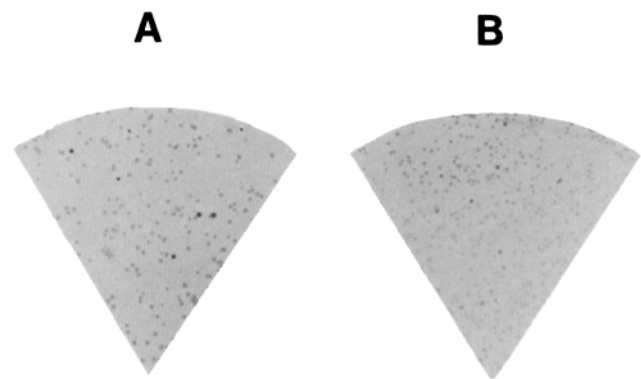
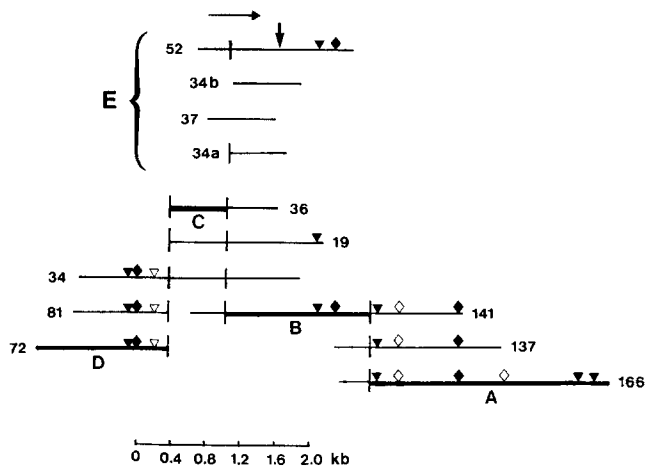


Figure 1. Detection of cDNA-encoded fusion proteins expressed in bacteriophage  $\lambda$ gt11 using an anti-MAP antiserum. A cDNA expression library was constructed in bacteriophage  $\lambda$ gt11 using polyA<sup>+</sup> mRNA from 8-d-old mouse brain. About  $5 \times 10^5$  plaques were screened using a rabbit anti-rat MAP antibody that detects both mouse MAP1 and mouse MAP2 (28). Seven clones were detected that were immunoreactive with the antiserum: three yielded a strong signal that proved to be due to MAP2 epitopes (23); the remaining four yielded weaker signals with the anti-MAP antibody. Examples of the immunoreactivity of one member of each class are shown at the penultimate purification step against a background of negative clones. (A) Strongly immunoreactive; (B) weakly immunoreactive.



**Figure 2.** Characterization of a series of overlapping cDNAs encoding the same contiguous mRNA molecule. Several overlapping cDNA clones were isolated by rescreening the  $\lambda$ gt11 cDNA library with the insert excised from the longest member of the set of clones expressing a weakly immunoreactive MAP fusion protein (*E*). Extended regions of cloned cDNA were then used in further screening experiments to produce the collection of cloned fragments shown in the figure. The transcriptional orientation (arrowed) was established by restriction mapping of the original expression clone in  $\lambda$ gt11 with respect to the lac Z promoter. The vertical arrow indicates the approximate position of the translational termination codon, determined from the size of the fusion proteins expressed by clone 52 (data not shown) and fragment *B*. Fragments *A*, *B*, *C*, and *D* were subcloned and used for the purposes described in the text.  $\nabla$ , *Sac*I;  $\blacklozenge$ , *Pst*I;  $\diamond$ , *Kpn*I;  $\nabla$ , *Bgl*II. Vertical bars denote *Eco*RI sites.

inserts as fusion proteins transcribed from the *E. coli* *trp* promoter and translated in all six reading frames. After induction with indole acrylic acid, the host cells were examined by SDS PAGE and immunoblotting for the presence of fusion proteins that were immunoreactive with the anti-MAP antiserum used to detect the original expression clones in the  $\lambda$ gt11 library. The result of this experiment is shown in Fig. 3I: in only one frame and orientation, cloned fragments *B* and *D* (Fig. 2) yielded fusion proteins of 48,000 D and 55,000 D, respectively, that were strongly immunoreactive with anti-MAP antibody. Since clone *B* and clone *D* are derived from the same mRNA species, this experiment demonstrates that a minimum of two MAP epitopes are recognized on the same molecule by our anti-MAP antiserum. Clone *A* yielded no immunoreactive fusion protein in any orientation. This result was expected, because based on the size of our MAP1 immunoreactive fusion proteins, and the direction of transcription of these expressing clones, we predicted that fragment *A* would represent entirely 3' untranslated region (see legend to Fig. 2).

To show that the immunoreactive fusion proteins expressed by clone *B* and clone *D* (Fig. 2) contained epitopes present in MAP1, but not MAP2, an immunoblot experiment was done using MAP1- and MAP2-specific antisera (23). The data (Fig. 3II) show that the fusion proteins encoded by both clone *B* and clone *D* are detected by the anti-MAP1-specific antiserum, but not the anti-MAP2 antiserum. On the basis of this evidence, the detection of multiple overlapping clones in the  $\lambda$ gt11 expression library, and the size and tissue distribution of the mRNA detected by the clones depicted in Fig. 2

(see below), we conclude that these sequences encode mouse MAP1.

### *Size and Tissue Distribution of MAP1 mRNA*

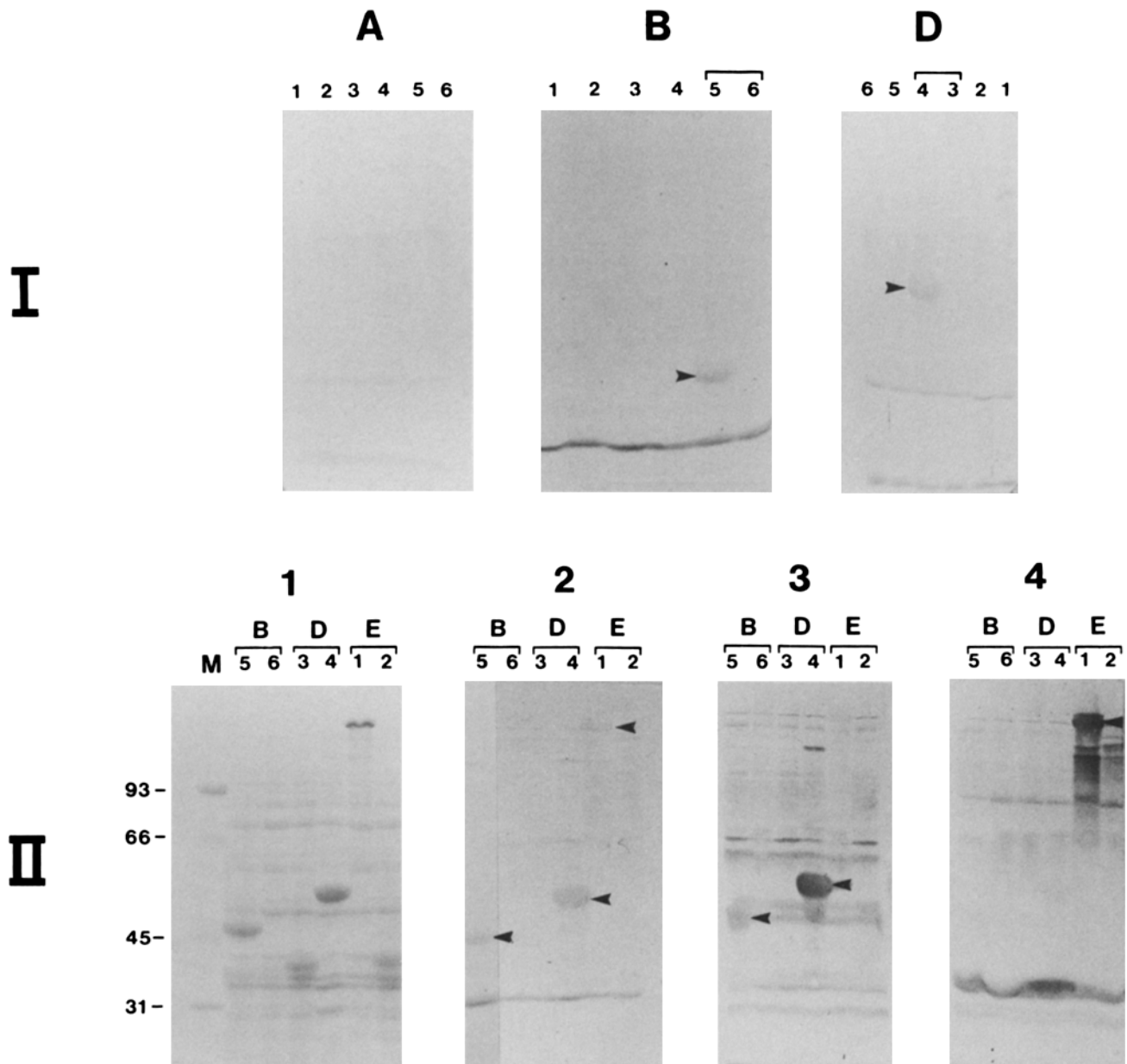
The size and heterogeneity (if any) of the mRNA detected by our cloned cDNAs were determined in an RNA blot transfer experiment in which total 8-d-old mouse brain RNA was probed with the excised inserts from subclones *B-D* (Fig. 2)  $^{32}$ P-labeled by nick-translation (26). A single band was observed (Fig. 4A) that showed some evidence of degradation. This pattern, which was reproducibly observed irrespective of the developmental age or method of preparation of the RNA, is probably a reflection of degradation *in vivo*, since neither MAP2 mRNA (23), neurofilament mRNA, glial filament mRNA, nor  $\alpha$ - or  $\beta$ -tubulin mRNAs detected in the same preparations showed any appreciable evidence of degradation (data not shown). In the absence of good high molecular weight RNA markers, the exact size of the MAP1 mRNA species was impossible to ascertain. The use of DNA size markers showed them to be essentially unresolved in the high molecular weight range on denaturing gels that contained formaldehyde, and in any case, they failed to run true with respect to ribosomal RNAs. On the basis of the migration of the MAP1 mRNA species relative to these imperfect markers, we estimate that the size of MAP1 mRNA is  $>10$  kb.

Previous studies using fluorescent MAP1-specific polyclonal and monoclonal antisera have detected MAP1 protein both as an abundantly expressed protein in brain (5, 6, 19, 20) and also in a variety of non-neuronal tissues and cell lines (7, 33). Such non-neuronal expression of MAP1 might have been observed as a consequence of cross-reactivity between epitopes present in non-MAP1 proteins. To detect the expression of MAP-1 at the mRNA level, an RNA blot transfer experiment was done in which total RNA from mouse brain, kidney, liver, stomach, spleen, and thymus was probed with our MAP1-specific cDNA probes. The data show abundant expression of the MAP1-specific mRNA in brain, with very much lower but nonetheless detectable levels in all the other tissues examined (Fig. 4B).

### *Regulation of MAP1 and MAP2 mRNAs in the Developing Mouse Brain and of MAP1 mRNA in Rat PC12 Cells in Response to NGF*

Previous studies using immunoblot analysis have revealed measurable changes in the levels of MAP1 and MAP2 in brain as a function of development. In particular, a marked increase in the abundance of MAP1 with increasing age has been reported (25). To see whether these changes were reflected at the mRNA level, total mRNA was prepared from the brains of mice of varying age and analyzed in an RNA blot transfer experiment using MAP1- and MAP2-specific cloned probes.  $\alpha$ - and  $\beta$ -Tubulin probes (10) were included in this experiment for comparison of developmental expression and relative abundance. Surprisingly, the result shows that both MAP1- and MAP2-specific mRNA decline somewhat from birth, the decline being much more pronounced in the case of MAP1 (Fig. 5).

Rat PC12 cells have been widely used as a model system of neuronal differentiation (15). Recently, two groups (14, 16) have reported a dramatic (10–20-fold) increase in the expression of MAP1 in these cells upon stimulation with NGF



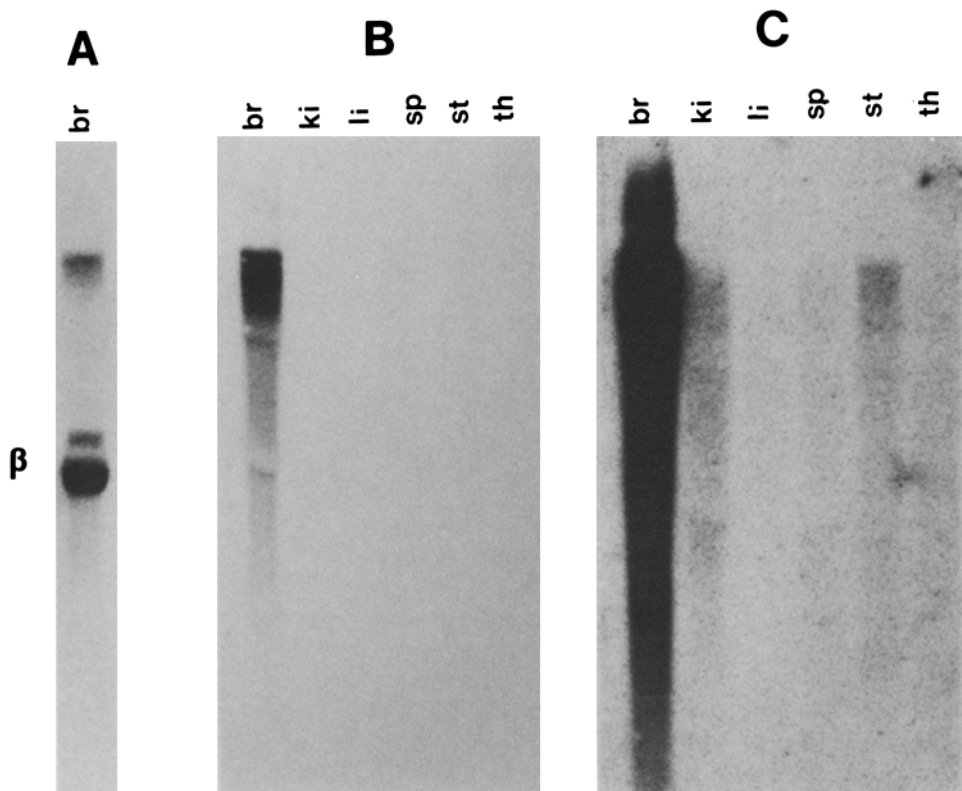
**Figure 3.** Two fusion proteins expressed by non-overlapping cDNAs encoding a contiguous mRNA each contain at least one MAP1-specific epitope. (I) *EcoRI* fragments A, B, and D (Fig. 2) were subcloned into inducible plasmid expression vectors (see text) in all six potential reading frames (lanes 1–6). The fusion proteins encoded by these constructs were then analyzed by immunoblotting of total *E. coli* lysates using in each case the anti-rat MAP antibody used to screen the  $\lambda$ gt11 expression library (28). (II) Two pairs of clones (from panel I, B5 and B6, D3 and D4) each pair containing inserts in opposite orientation and expressing an immunoreactive fusion protein in one of these orientations, were further analyzed along with an equivalent pair of MAP2 expression clones (23) as a control (tracks E1 and E2). (1) Coomassie Blue-stained gel of *E. coli* lysates. (2–4) Immunoblots of duplicate gels stained with (2) the anti-rat MAP antibody (which recognizes both MAP1 and MAP2) used to screen the expression library (28), (3) the anti-mouse MAP1-specific antibody (23), and (4) the anti-mouse MAP2-specific antibody (23). Immunoreactive fusion proteins are arrowed. M, molecular size markers (in kilodaltons). The high molecular weight, immunoreactive species uniquely present in track II.3.D4 is ascribable to a fusion protein encoded by fragment D, which mutated in the host bacteria expressing this fragment to give the shorter and more abundant fusion protein evident in the same track.

concomitant with the characteristic outgrowth of neurites. This increase was reversed when NGF was removed and the neurites disappeared. Because the mouse MAP1-specific probes described here cross-hybridize with corresponding sequences in rat (see below), we were able to test whether these reported NGF-induced alterations in the level of MAP1 protein in rat PC12 cells were accompanied by an increase in the abundance of MAP1-specific mRNA. Total RNA was pre-

pared from PC12 cells at various times after their exposure to NGF. The result (Fig. 6) shows only a moderate change in the level of MAP1-specific sequences; MAP2 is undetectable in these cells.

#### *Clones B, C, and D Detect a Single Gene in Mouse*

To determine the number of genomic sequences homologous to our MAP1-specific probes, a genomic Southern blot exper-



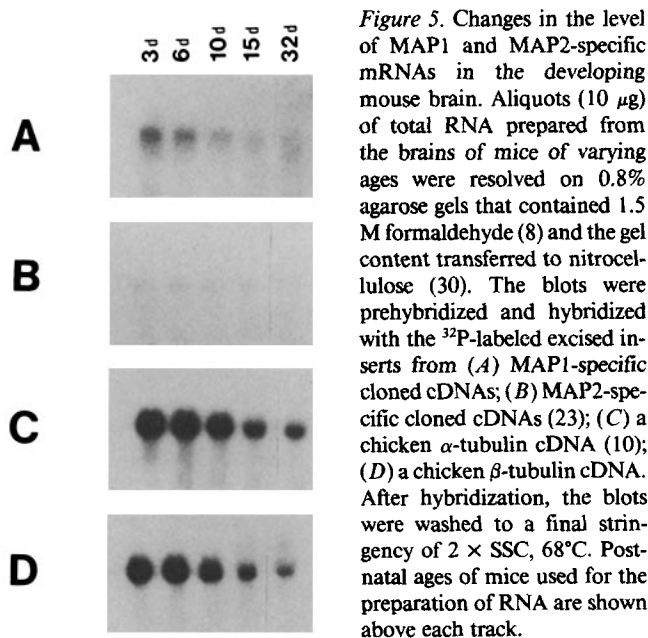
**Figure 4.** A single mRNA encoding mouse MAP1 and its tissue-specific expression. Aliquots of RNA prepared from mouse tissues were resolved on an 0.8% denaturing agarose gel (8), and the gel content transferred to nitrocellulose (30). (A) RNA (10  $\mu$ g) from 15-d-old mouse brain probed with the excised insert from clone 52 (Fig. 2) and a chicken  $\beta$ -tubulin cDNA (10)  $^{32}$ P-labeled by nick-translation (26).  $\beta$ , 2.2-kb and 1.8-kb  $\beta$ -tubulin mRNAs. (B and C) PolyA<sup>+</sup> mRNA (15  $\mu$ g) from 32-d-old mouse brain, kidney, liver, spleen, stomach, and thymus probed as in A, but without the chicken  $\beta$ -tubulin cDNA. (B) 12-h exposure; (C) 20-d exposure (both with an intensifying screen). After hybridization in 50% formamide, 5  $\times$  SSC, 42°C, blots were washed to a final stringency of 2  $\times$  SSC, 68°C.

iment was done (30) using whole mouse DNA digested to completion with either *Bam*HI or *Kpn*I. These enzymes were chosen for this experiment because the non-overlapping cloned fragments B, C, and D (Fig. 2) encoding MAP1 lack recognition sites for these enzymes. The blot was hybridized and washed under low stringency conditions (Fig. 7), so as to allow the potential detection of mismatched sequences bearing some homology to the cDNA probes in addition to the homologous genomic sequences. The result shows that, irrespective of the final stringency conditions, the non-overlap-

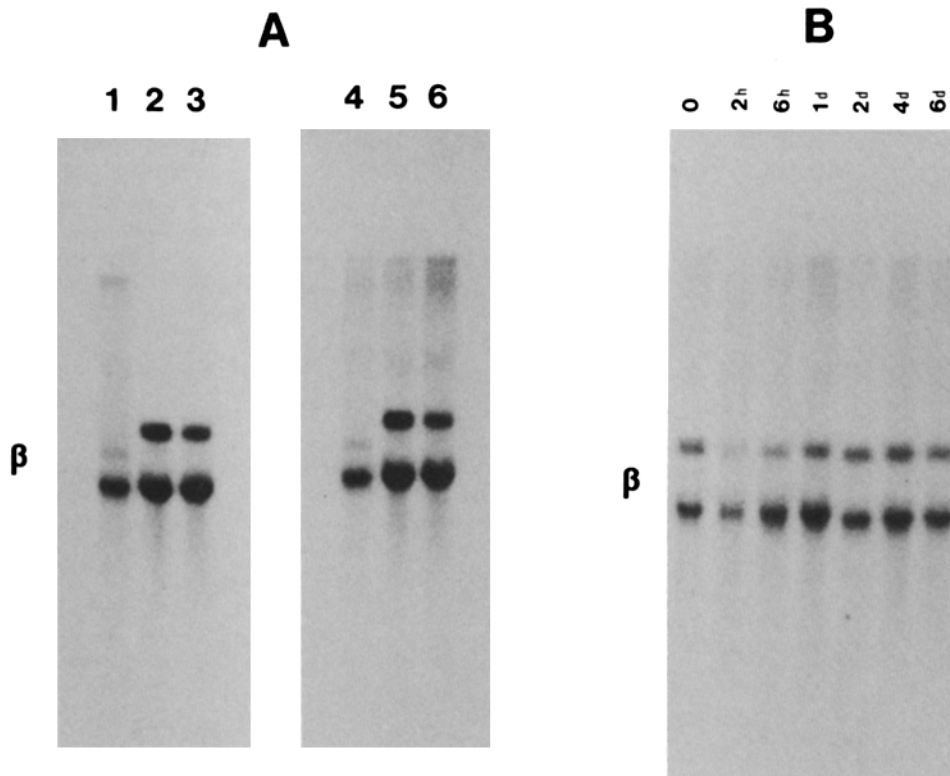
ping cloned cDNAs B, C, and D detect a single restriction fragment in either digest (Fig. 7); clone A, which consists of 3' untranslated region sequences (Fig. 2), detects a continuum of hybridizing fragments consistent with hybridization to repetitive sequences (data not shown). These data therefore demonstrate the existence of a single mouse gene corresponding to clones B, C, and D, that is not a member of a multigene family related by detectable homology at the DNA level.

**Sequences Encoding Mouse MAP1 Cross-hybridize with Sequences in Rat, Human, and Chicken DNA, But Not with DNA from Frog, Drosophila, or Sea Urchin**

The ability of anti-MAP1 antisera to detect epitopes across species boundaries (7, 33) implies the interspecies conservation of at least some MAP1-specific sequences. However, these experiments do not provide a measure of the extent of interspecies conservation, and, in any case, the possibility of interspecies cross-reactivity of related epitopes on non-MAP1 proteins cannot be ruled out. To investigate interspecies homologies at the DNA level, a genomic Southern blot experiment was done using DNA from mouse, rat, human, chicken, frog, sea urchin, and *Drosophila* (lanes 1-7). The data show that, under conditions of low stringency, the mouse MAP1 probe detects two bands in rat, and a single band in human and chicken DNA, but not in the genomes of the other species examined (Fig. 8). The cross-reactivity with rat and human, but not with chicken DNA, remained when the blot was rewashed to high stringency (0.2  $\times$  150 mM NaCl, 15 mM Na citrate [SSC], 68°C) (data not shown). The background hybridization evident in frog and sea urchin DNA at 2  $\times$  SSC, 42°C (lanes 5 and 6) also disappeared at higher stringency. Thus, while there seems to be considerable conservation of

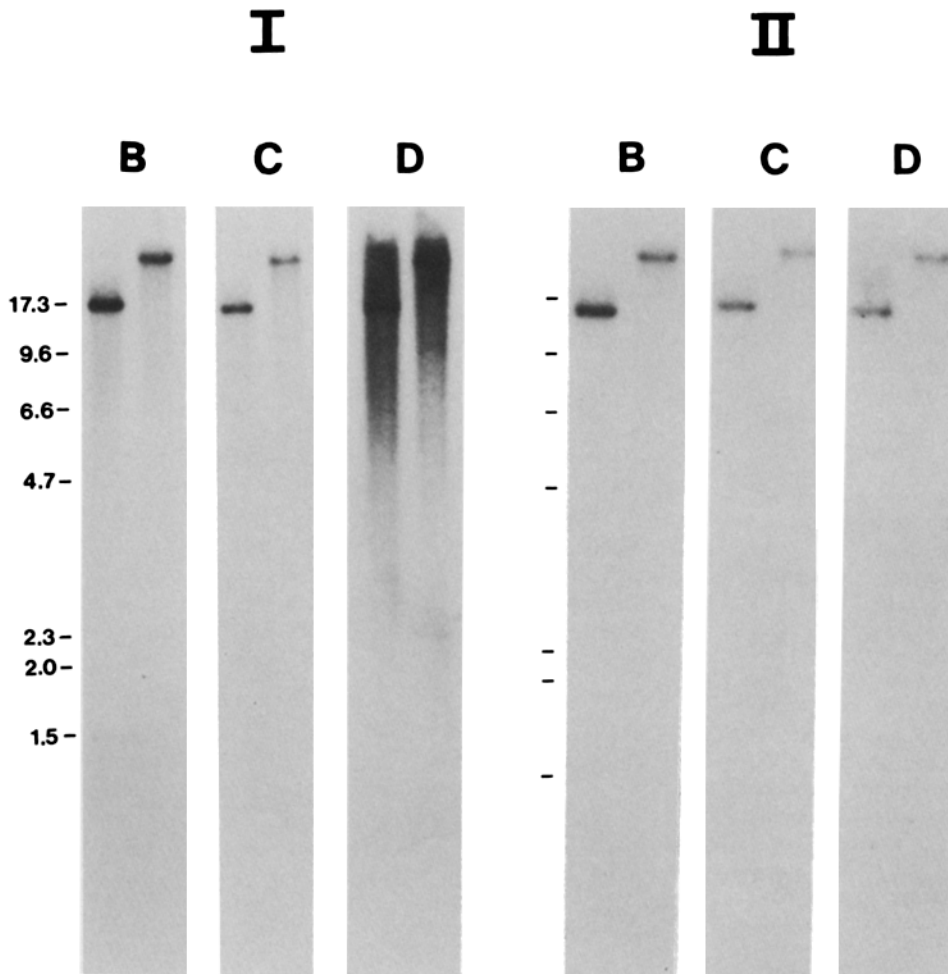


**Figure 5.** Changes in the level of MAP1 and MAP2-specific mRNAs in the developing mouse brain. Aliquots (10  $\mu$ g) of total RNA prepared from the brains of mice of varying ages were resolved on 0.8% agarose gels that contained 1.5 M formaldehyde (8) and the gel content transferred to nitrocellulose (30). The blots were prehybridized and hybridized with the  $^{32}$ P-labeled excised inserts from (A) MAP1-specific cloned cDNAs; (B) MAP2-specific cloned cDNAs (23); (C) a chicken  $\alpha$ -tubulin cDNA (10); (D) a chicken  $\beta$ -tubulin cDNA. After hybridization, the blots were washed to a final stringency of 2  $\times$  SSC, 68°C. Post-natal ages of mice used for the preparation of RNA are shown above each track.

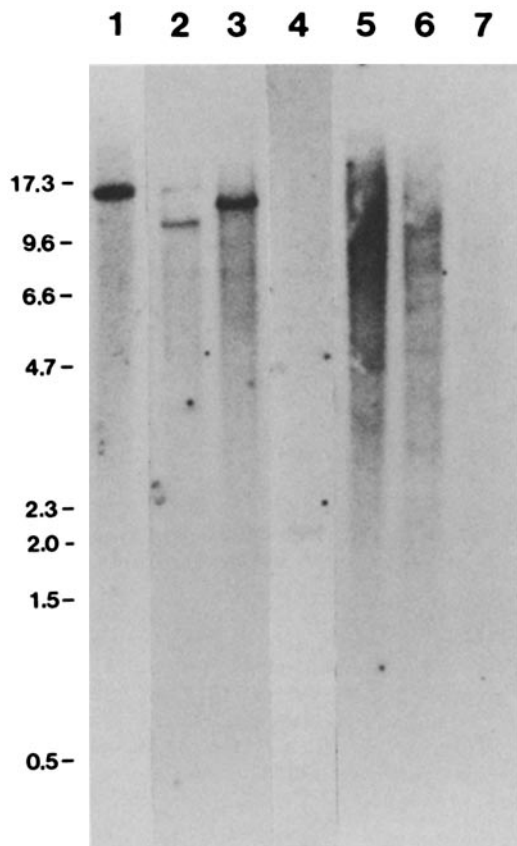


**Figure 6.** Moderate elevation in MAP1-specific mRNA in NGF-induced PC12 cells. (A) Aliquots (10  $\mu$ g) of RNA from rat brain (lanes 1 and 4) and from PC12 cells either unexposed (lanes 2 and 5) or exposed for 10 d (lanes 3 and 6) to NGF were resolved on a 0.8% agarose gel that contained 1.5 M formaldehyde (8) and the gel content transferred to nitrocellulose (31). The blots were hybridized with either the excised  $^{32}$ P-labeled insert from clone 56 (encoding MAP2, reference 23) (lanes 1–3) or with the  $^{32}$ P-labeled insert from clone 52 (encoding MAP1, Fig. 2) (lanes 4–6). In each case, the probes were mixed with the  $^{32}$ P-labeled insert from a chicken  $\beta$ -tubulin cDNA (10) to provide internal size markers and as a check on the integrity of the mRNA preparations. (B) Aliquots (10  $\mu$ g) of total RNA from PC12 cells exposed to NGF for the times shown in the figure were resolved in an 0.8% denaturing agarose gel (8) and the gel content transferred to

nitrocellulose (30). The blot was prehybridized, and hybridized with the same probes used in A, lanes 4–6. All blots were washed to a final stringency of  $2 \times$  SSC,  $45^\circ\text{C}$ .  $\beta$ , 1.8-kb, 2.2-kb, and 2.6-kb  $\beta$ -tubulin mRNAs.



**Figure 7.** MAP1-specific cDNA clones detect a single copy gene in mouse. Aliquots (5  $\mu$ g) of mouse genomic DNA were digested with *Bam*HI (left hand lanes) or *Kpn*I (right hand lanes), resolved on a 0.8% agarose gel, and the gel content transferred to nitrocellulose (30). Separate blots were probed with MAP1 cDNA fragments B, C, and D (Fig. 2)  $^{32}$ P-labeled by nick-translation (26). So as to allow the detection of possible homologous but mismatched sequences in the genomic DNA, the blots were hybridized (in 40% formamide,  $5 \times$  SSC,  $42^\circ\text{C}$ ) and were initially washed to low stringency ( $2 \times$  SSC,  $42^\circ\text{C}$ ) (panel I), and then to high stringency ( $0.2 \times$  SSC,  $68^\circ\text{C}$ ) (panel II). An intense continuum of labeling was detected by fragment A (Fig. 2) which remained after washing to high stringency (data not shown).



**Figure 8.** Limited interspecies conservation of a MAP1 gene. Aliquots (5  $\mu$ g) of genomic DNAs were digested with *Hind*III, the fragments resolved on a 0.8% agarose gel, and the gel content transferred to nitrocellulose (30). The blot was prehybridized and hybridized with the mixed probes *B*, *C*, and *D* and washed as described in the legend to Fig. 7, panel *I*. Lanes 1–7, DNA from mouse, rat, human, chicken, frog, sea urchin, and *Drosophila*. Molecular size markers (in kb) are shown at the left.

MAP1 genomic sequences within mammalian species, this conservation does not extend to invertebrate species.

## Discussion

In the accompanying paper (23), we describe the isolation of a series of overlapping cloned cDNAs encoding mouse MAP2. These clones were detected as a result of the expression of immunoreactive fusion proteins in the host by bacteriophage  $\lambda$ gt11. Since the antiserum used in the screening experiments (28) detected both MAP1 and MAP2 (23), the detection of a second set of clones that did not cross-hybridize with the MAP2-specific cDNAs but that nonetheless expressed weakly immunoreactive fusion proteins (Fig. 1 *B*), suggested the possibility that they might encode mouse MAP1. To explore this possibility, a number of experimental approaches were undertaken. First, a series of cDNA “walking” experiments was done that served both to extend the region of cloned DNA, as well as provide cloned fragments that were contiguous but non-overlapping with respect to the original cloned cDNA. Expression of these non-overlapping cloned fragments in an inducible plasmid vector yielded fusion proteins that were immunoreactive with a polyclonal anti-MAP1-specific anti-

body, but not with a polyclonal anti-MAP2-specific antibody (Fig. 3). Thus, that portion of the mRNA species represented by the series of overlapping cloned cDNAs shown in Fig. 2 encodes a minimum of two MAP1-specific epitopes. The statistical probability of detecting a single polypeptide containing two epitopes both adventitiously recognized by a MAP1-specific antiserum is vanishingly small. We conclude, therefore, that the series of cDNA clones shown in Fig. 2 encodes mouse MAP1. This conclusion is consistent with the very large size of the mRNA molecule detected by these cDNA probes, and its tissue distribution, discussed below.

The cDNA probes shown in Fig. 2 detect an mRNA species of >10 kb in mouse brain. The mRNA appears as a pronounced band together with a smear of hybridizing sequences of lower size. This continuum does not seem likely to be a consequence of mRNA degradation during the isolation procedure, since MAP2 mRNA (which is only slightly smaller in size) is largely intact in the same RNA preparations (23). Rather, it appears that there are significant quantities of partially degraded MAP1 mRNA *in vivo*, and that these molecules co-purify with intact mRNA. The large size of the mRNA detected by our cloned probes is consistent with the coding requirement for MAP1: though the exact size of MAP1 polypeptides is uncertain, an  $M_r$  of 350,000 would require a coding capacity of  $\sim$ 9.5 kb. The approximate location of the stop codon is indicated in Fig. 2, as deduced from the size of the original  $\lambda$ gt11 fusion proteins and their direction of transcription. Thus, about half of the region covered by our cDNA clones corresponds to coding sequences, and half to 3' untranslated region.

Previous studies using MAP1-specific antibodies have identified MAP1-cross-reactive epitopes in several tissues other than brain (33) as well as in a wide variety of tissue culture cell lines (7, 33). The tissue distribution of MAP1 mRNA detected by our cloned cDNA probes (Fig. 4) is consistent with these observations, though the level of expression (relative to that in brain) is exceedingly low. Indeed, it is conceivable that the very weak signals obtained in blots containing RNA from non-neuronal tissues could in at least some cases be a result of MAP1-specific mRNA contained exclusively in innervating cells associated with that tissue. In any event, quantitative comparison of the MAP1-specific signals detected in brain RNA with MAP1-specific signals detected in non-neuronal tissue shows that they differ by a factor of at least 500, and the level of MAP1 expression in tissues other than brain is therefore exceedingly low.

The abundance of MAP1- and MAP2-specific mRNAs declines slightly in the developing mouse brain, the decline being much more pronounced in the case of MAP1 (Fig. 5). Moreover, there was only a slight change in the level of MAP1-specific mRNA in rat PC12 cells upon exposure to NGF (Fig. 6). These results are rather surprising in view of the reported increase in the level of MAP1 that occurs in the developing postnatal rat brain (5), and the dramatic rise in MAP1 in NGF-induced rat PC12 cells (14, 16). Such increases could be explained by regulation of MAP1 at the translational or posttranslational level. In this context, it is interesting to note that the microtubule associated proteins  $\tau$  appear to be regulated in this fashion (13). To test this hypothesis, we measured changes in the level of MAP1 protein in PC12 cells exposed to NGF in an immunoblotting experiment using the poly-



clonal antisera described in the accompanying paper (23). In contrast to the reported dramatic increase observed using an anti-MAP1 monoclonal antibody (14), we found little detectable increase in MAP1 levels (data not shown). This discrepancy could be explained by differences in the epitopes recognized by the monoclonal and polyclonal antisera; for example, the monoclonal antibody could be specific for an epitope conferred on the protein by phosphorylation.

On low percentage SDS polyacrylamide gels, MAP1 is resolvable into three distinct subcomponents, MAP1a, MAP1b, and MAP1c (6, 7). While these polypeptides are to some extent immunologically and biochemically distinct, the genetic relationship between them is unclear. Genomic Southern blot analysis using cloned cDNAs corresponding to the coding region of MAP1 detected a single restriction fragment in digests of mouse DNA, even under conditions of low stringency ( $2 \times \text{SSC}$ ,  $42^\circ\text{C}$ ) (Fig. 7). Thus, the gene encoding MAP1 is not a member of a multigene family, at least as defined by the ability of family members to cross-hybridize at the DNA level. The single copy gene detected by the cDNAs characterized in this work could encode either MAP1a, MAP1b, or MAP1c, or, by a process of differential splicing of alternative exons, it could encode two or conceivably all three of these polypeptides. An alternative splicing mechanism has been shown to operate, for example, in the generation from a single gene of several related but distinct fibronectin (27) or calcitonin (1) polypeptides. In this context, it is interesting to note that one of the cDNA expression clones, clone 37, isolated from  $\lambda\text{gt}11$  library (see Fig. 1 of accompanying paper) differs from others spanning the same region in that it lacks an *EcoRI* restriction site (Fig. 2). Experiments are currently directed towards establishing whether this restriction site difference reflects a polymorphic variation. Alternatively, clone 37 might lack this site because it lacks DNA sequences corresponding to an exon that is encoded by other overlapping clones.

The MAP1-specific cDNA clones described here cross-hybridize under high stringency conditions ( $0.2 \times \text{SSC}$ ,  $68^\circ\text{C}$ ) with rat and human genomic DNA, but not with DNA from any other non-mammalian source tested (Fig. 8). Thus, in contrast to  $\alpha$ - and  $\beta$ -tubulin, both of which are extensively evolutionarily conserved (10), there would appear to be considerable species divergence in the sequences encoding MAP1. This could reflect either the absence of strong selective constraints over the region of the protein encoded by our cDNAs, or some divergence of MAP1 function among different species. A similar lack of interspecies sequence conservation applies in the case of MAP2 (23).

While the genetic basis of MAP1 heterogeneity and the precise biological function of MAP1 remain uncertain, the isolation of cloned probes provides a starting point for a molecular and functional analysis of these proteins at the nucleic acid level. In particular, it should be possible to use these probes inserted into appropriate vectors (22) for the extinction of MAP1 gene expression, and to determine the effect of such targeted elimination of MAP1 on cell growth and neuronal differentiation.

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