Analysis of the Primary Sequence and Microtubule-binding Region of the *Drosophila* 205K MAP

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Abstract. We have sequenced cDNA clones encoding the *Drosophila* 205K microtubule-associated protein (MAP), a protein that may be the species specific homologue of mammalian MAP4. The peptide sequence deduced from the longest open-reading frame reveals a hydrophilic protein, which has basic and acidic regions that are similar in organization to mammalian MAP2. Using truncated forms of the 205K MAP, a 232-amino acid region could be defined that is necessary for microtubule binding. The amino acid sequence of this region shares no similarity with the binding motif of MAP2 or tau. We also analyzed several embryonic cDNA clones, which show the existence of differentially spliced mRNAs. Finally, we identified several potential protein kinase target sequences. One of these is distal to the microtubule-binding site and fits the phosphorylation consensus sequence of proteins phosphorylated by the mitosis specific protein kinase *cdc2*. Our data suggest that the 205K MAP uses a microtubule-binding motif unlike that found in other MAPs, and also raise the possibility that the activities of the 205K MAP may be regulated by alternative splicing and phosphorylation.

THE regulation of microtubule assembly and interaction is essential for proper cell morphology and cell division. A class of proteins known as microtubule-associated proteins (MAPs;[1] reviewed in Olmsted, 1986) may play an important role in such regulation. MAPs from different species have been generally identified and isolated by virtue of their copurification with tubulin. Those MAPs that have been examined share the ability to stimulate the assembly of microtubules from tubulin dimers in vitro (Cleveland et al., 1977; Murphy et al., 1977; Bulinski and Borisy, 1979). An additional property of many MAPs is that they are generally found in association with microtubules in fixed cells labeled with anti-MAP antibodies (Bernhardt and Matus, 1984; Bloom et al., 1984; Parysek et al., 1984*a*,*b*).

While the distribution of different MAPs is well documented, the molecular structure of only a few of them has been elucidated. A MAP of 320 kD has been isolated from trypanosomes as a membrane cytoskeletal component. The amino acid sequence of this protein consists of >50 nearly identical tandem repeats of 38 amino acids (Schneider et al., 1988). A group of well-characterized MAPs isolated from mammalian brain consists of tau, MAP1B, and MAP2. The genes for MAP2 (Lewis et al., 1988), tau (Lee et al., 1988; Goedert et al., 1988; 1989), and MAP1B (Noble et al., 1989) have been cloned and their primary sequence has been determined. Both tau and MAP2 contain an 18-amino acid peptide repeat motif, which has been shown to be sufficient for microtubule binding (Himmler et al., 1989; Lewis et al., 1988). MAP1B has a different repeated motif responsible for microtubule binding (Noble et al., 1989). Electron microscopic studies have demonstrated that MAP1B is an extended molecule that is ~200 nm long (Sato-Yoshitake et al., 1989); and that MAP2 forms rod-like projections of 100–200 nm attached to the microtubule surface (Voter and Erickson, 1982). Similarly, tau exists as a 40–60-nm-long extended molecule, which also can form projections off of microtubules (Hirokawa et al., 1988).

A major MAP identified in *Drosophila*, the 205K MAP (Goldstein et al., 1986), was found to be colocalized with mitotic spindle and cytoplasmic microtubules in immunofluorescence staining experiments of cultured cells. This protein is likely to be a member of the MAP4 family, which appears to consist of mouse MAP4 (Olmsted and Lyon, 1981), the HeLa 210K MAP (Bulinski and Borisy, 1979), and the bovine 190K MAP (Murofushi et al., 1986). These MAPs share several properties including possession of various isoforms, a molecular mass ~200 kD, and thermostability. Besides their biochemical similarities, some members of the MAP4 family, including the *Drosophila* 205K MAP, have been shown to be immunologically related (Kotani et al., 1987, 1988; West, R. R., K. M. Tenbarge, M. Gorman, L. S. B. Goldstein, and J. M. Olmsted. 1988. *J. Cell Biol.* 107:460a). In most cases, little information is available as to the structure and function of the MAP4 proteins. However, studies on the structure of the HeLa 210K MAP (Bulinski and Borisy, 1980) led to the conclusion that it exists as an extended molecule that forms dimers in solution.

The best evidence for a functional role of proteins of the

1. *Abbreviations used in this paper*: MAP, microtubule-associated protein; ORF, open reading frame.

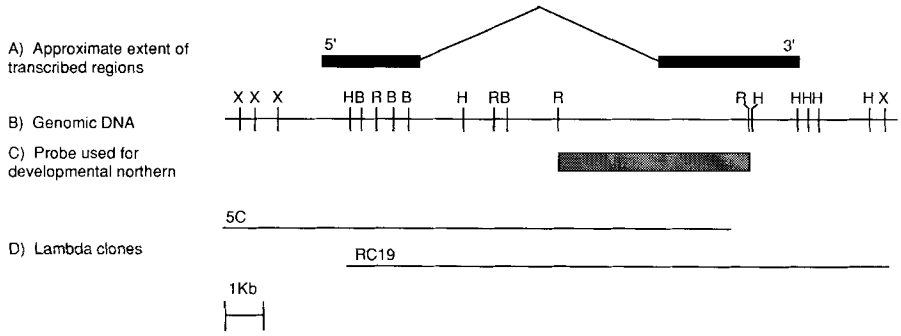

Figure 1. Organization of the 205K MAP gene. *X*, Xba I; *H*, Hind III; *B*, Bam HI; *R*, Eco RI. The position in the genomic DNA of the small alternative exons defined by restriction mapping and sequencing is unknown and therefore not indicated.

MAP4 family in vivo comes from injections of mAbs recognizing the HeLa 210K MAP into dividing cells. This treatment resulted in mitotic arrest (Izant, 1981; Izant et al., 1983). From these experiments it can be concluded that the HeLa 210K MAP is directly, or indirectly, involved in microtubule behavior during mitosis.

To understand better the structure and possible functions of the MAP4 family and the *Drosophila* 205K MAP, we have determined the primary sequence of the 205K MAP, identified alternatively spliced variants, and defined the region of the protein that binds microtubules. These investigations suggest that an additional motif, not found in other microtubule-binding proteins, may mediate the interaction of the *Drosophila* 205K MAP with microtubules. Our investigations further suggest that this putative regulatory protein may itself be regulated by phosphorylation and alternative splicing.

Materials and Methods

General molecular biology methods such as subcloning, Northern and Southern blotting, and restriction mapping were carried out as described in Maniatis et al. (1983).

Isolation and Characterization of Genomic and cDNA Clones

Genomic clones were isolated from the Maniatis library (Maniatis et al., 1978) and characterized by restriction mapping. The cDNA clones were isolated from the 4–8-h embryonic cDNA libraries described by Brown et al. (1989). We isolated 22 clones after screening of 1×10^6 colonies with a 1.8-kb subclone of a previously isolated (Goldstein, unpublished) cDNA clone that corresponded to the 5′ 40% of the coding sequence as defined by Northern analysis. All cDNA clones were restriction mapped using Eco RI, Bam HI, and Hind III. This process and the observed size of the inserts allowed us to assign the extent of the coding sequence that each cDNA contained by comparison to the genomic restriction map and the putative transcription unit borders assigned by Northern analysis. The longest cDNA clones were near the total size of the major mRNA species observed. In the course of analyzing these cDNA clones, we observed that 4 of them (out of 14)) that extended past the 5′ Eco RI site had different sized internal Eco RI fragments than the clone, B3. Further restriction analysis localized the differences to the Pst I–Hinc II fragment indicated in Fig. 1. This was the region sequenced in the cDNA clones J5 and C2, and that defined the alternatively spliced forms reported in this study.

Generation of Deletion-bearing cDNA Clones

As described in Results, cDNA clones B3, C2, J5, and G4 were originally isolated from a 4–8-h embryonic cDNA library in pNB40 (Brown et al., 1989). We used a cDNA that was full-length with respect to the protein coding sequence for the generation of all deletion bearing cDNA clones. This construct, B3-SK, was generated in the Bluescript vector and consists of a 600-bp Hind III–Sal I fragment at the 5′ end, derived from cDNA G4, and a 4.1-kb Sal I–Not I fragment derived from cDNA B3. cDNA G4 is 400 bp shorter at its 5′ end than cDNA B3 but the DNA sequence, and hence the protein sequence, is identical in both cDNAs in this region. The orientation of the insert into the Bluescript vector allowed transcription from the T3 promoter. Nested deletions were generated in cDNA clone B3-SK with exonuclease III by using the Erase-a-Base kit (Promega Biotec, Madison, WI).

DNA Sequencing of B3

All deletion-bearing clones were sequenced using double-stranded DNA. A Sequenase kit (U.S. Biochemical Corp., Cleveland, OH) was used for the dideoxy sequencing reactions (Sanger et al., 1977). Both strands were sequenced independently and each basepair was sequenced at least four times. The DNA sequence was assembled and analyzed using the Bionet national computer facility and the UWGCG sequence analysis package (Devereux et al., 1984).

Microtubule Binding Assay

The cDNA clone B3 and deletion-bearing cDNA clones, derived from B3-SK, were transcribed in vitro using SP6 or T3 RNA polymerases. In vitro transcription and translation in a reticulocyte lysate were carried out as described by Yang et al. (1989). In vitro translated [^{35}S]methionine-labeled proteins bearing truncations or internal deletions were tested for microtubule-binding activity. 1–2 μl of a reticulocyte lysate translation mix was incubated for 30 min at 22°C with 8 μg of taxol-stabilized microtubules from bovine brain (a gift of Dr. Jon Scholey, University of California, Davis) in assembly buffer (0.1 M Pipes, 2 mM EGTA, 1 mM $MgCl_2$, 20 μM taxol, 2 mM GTP, 2 mM DTT, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 μg/ml aprotinin, 1 μg/ml TAME (*Nα-p*-tosyl-L-arginine methyl ester), and 1 mM PMSF). After incubation, the mixtures were subjected to centrifugation at 50,000 g_{max} for 30 min. The resulting pellet and supernatant fractions were analyzed on 7.5% SDS-polyacrylamide gels. The gels were stained with Coomassie blue, fixed, enhanced with Enlightening solution (Dupont Corp., Wilmington, DE), and then dried and exposed to x-ray film.

Developmental Northern Analysis

Timed embryo, larva, and pupa collections were collected and RNA prepared as described in St. Johnston et al. (1990). 3.5–5 μg poly A^+ RNA was run on 0.8% formaldehyde agarose gels, transferred, probed, and hybridized as previously described (Goldstein et al., 1986).

Results

Organization of the 205K MAP Gene

Previous work resulted in the isolation of small segments of the 205K MAP gene (Goldstein et al., 1986). To recover and ultimately sequence cDNA clones, we mapped the extent and location of the 205K MAP transcription unit relative to the genomic DNA. We began by isolating several overlapping genomic clones in the 205K MAP gene, first by hybridization to 205K MAP coding sequences and then by walking from the ends of the initial phage isolated. After restriction

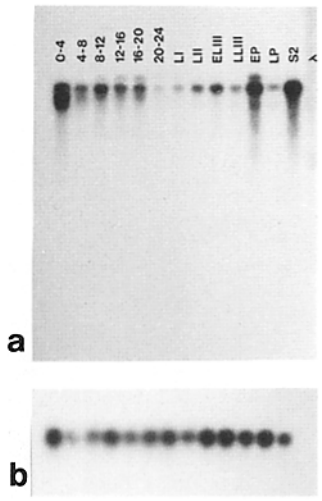

Figure 2. Developmental Northern analysis. 3.5 μg poly A^+ RNA extracted from embryos of different stages (0–4, 4–8, 8–12, 12–16, 16–20, and 20–24), as well as from larval stages I, II, early III, and late III, early and late pupal stage and Schneider S2 cells, was loaded onto each lane. The hybridization probe in *A* was a genomic DNA fragment containing the 5-kb Eco RI fragment indicated in Fig. 1 of the coding region and in *B* the filter was reprobed for ribosomal protein 49 mRNA.

mapping of each of the phage, most Eco RI fragments were subcloned, and used as probes in Northern blot analysis to define the approximate extent of the transcription unit. Restriction mapping of cDNA clones (see Materials and Methods) recovered from a 4–8-h embryonic library (Brown et al., 1989), further refined the map of this gene. The approximate position of the 5′ end is supported by nuclease protection and primer extension analysis (Laymon, R., and L. Goldstein, unpublished). The results are summarized in Fig. 1.

Developmental Northern Analyses

To ascertain the developmental times at which the 205K MAP was expressed, and to determine whether there was a set of grossly different RNA species expressed during development, we examined the 205K MAP RNA species present at different times during development (see Fig. 2). To control for differences in RNA loading, we probed with a clone for a ribosomal protein (rp49; O'Connell and Rosbash, 1984). Using probes from throughout the entire 205K MAP coding region, we found that 205K MAP sequences are present at most times during development, with a minimum at second larval instar, and a maximum at 4–8 h of development. Although the predominant species migrates at a mobility corresponding to 5.5–5.7 kb, many alternative RNA species were also observed. These species are all homologous to the same strand as the previously known 205K MAP mRNA species (data not shown), and all of them have various expression profiles relative to the major 205K MAP mRNA species. Whether these alternative mRNA species encode alternative protein isoforms remains to be determined.

DNA Sequence of cDNA Clone B3

To elucidate the primary structure of the 205K MAP, the entire DNA sequence of a 4.7-kb cDNA clone, B3, was determined (see Fig. 3 *A*). The cDNA clone B3 is ~700 bp shorter than the size of the major mRNA observed in Northern blot analysis. Our primer extension analyses and nuclease protection analyses indicate that the 5′ end of cDNA B3 is coterminal with a major 6–12-h embryonic and cell culture RNA species (Laymon, R., and L. Goldstein, unpublished observations). Restriction mapping suggests that the shorter size of B3 may result from truncation at the 3′ end in the 3′ untranslated region (Gorman, M., and L. Goldstein, unpublished observations). Whether this results from internal priming during cDNA synthesis or utilization of more than one polyadenylation sequence has not been determined. In vitro transcription and translation of the B3 cDNA clone resulted in a protein that comigrated with native 205K MAP (not shown). This latter observation constitutes strong evidence that this cDNA clone contains the entire protein coding region of the 205K MAP gene.

Surprisingly, the DNA sequence of cDNA clone B3 contains a much shorter protein coding sequence than expected for a protein of ~200 kD. The nucleotide sequence of B3 is 4,639 nucleotides long and contains only one long open-reading frame (ORF) of 3,489 nucleotides. This ORF is predicted to encode 1,163 amino acids or a protein of 130 kD from the first in frame methionine to the stop codon. Other ORFs are much smaller in size and are therefore unlikely to encode a large protein. All three reading frames contain stop codons upstream from the predicted translation start codon at nucleotide position 421 and downstream from the stop codon of the longest ORF at nucleotide position 3910. The first in frame methionine initiation codon does not appear to be surrounded by a sequence that fits the *Drosophila* initiation consensus sequence (Cavener, 1987). The second in frame methionine codon at nucleotide position 1030 does not fit the initiation consensus sequence either, and would result in a protein that is 203 amino acids shorter. A 5′ deletion-bearing cDNA that lacks the first ATG and starts at the Sal I site at position 585 was not translatable in reticulocyte lysates. On the contrary several plasmid constructs containing the first ATG, but which were deleted of the second, were translated, indicating that at least in the reticulocyte lysate system the first ATG works as a better translation initiation codon than the second. Therefore, we feel confident that the first ATG represents the translation start site. The stop codon at nucleotide position 3910 is followed by multiple stops in all three frames. The 721-bp 3′ untranslated sequence is very AT rich and ends with a poly A tract that is preceded by a polyadenylation consensus sequence AATAAA (Wickens and Stephenson, 1989) at nucleotide position 4177.

Amino Acid Sequence of the 205K MAP

We analyzed the amino acid sequence of the 205K MAP to define characteristic features of the protein that might suggest specific functions. We examined the amino acid composition, charge distribution, similarity to other proteins, and possible secondary structures.

Although the amino acid composition of the 205K MAP is not exceptional, its distribution of charges is (Fig. 4). Interestingly, the distribution of charged amino acids divides the protein into acidic and basic domains. The region from amino acid residue 1 to 762 predicts a protein with an estimated pI of 4.0, whereas the region from 763 to 1102 predicts a protein with a calculated pI of 12.9. The 61 amino acids of the carboxy-terminal end have a predicted pI of 4.4.

We also calculated the average charge distribution for two other MAP sequences that are available, MAP2 (Lewis et al., 1988) and tau (Lee et al., 1988). For MAP2 we found

A

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CCCCCCCCCCAAAAAAAAATTAGTCGGCAAGCAATTCAAAAGTAAGAGCAACCGGAGCGA      60

ACAAAAAAGGGATAACTTACTAAAATCTCTGAAAGAAAAATAAAAAGACTAACGGGCTCG     120

GCAAGCTGTGTTTATTTCGACAAGTAATTATATACTTGGAGTGCAAGCAAAGGCGAAGGA     180

AGTGGAAGAAGGACAAGCAACGAAATCGTGCTCTTATCCGTTCCTGTACTGTGTCTCTCT     240

TTCGCTGGGAGAGTGTGTGTATTGGTGTGAAGTGTAGAAATCTGCAAGAACAGCAACGCC     300

AATAAAAGTGGAATCGAGAAAAAAAGCAGTGGCGCGTGAATCACGAGCAATCTGAATCAT     360

CTCTCTACAAAAATACCTGTTTCTGTTGGCGCATCATTTATACCAATTAAATCCTAAAGG     420

ATGGAACACCACGAGGACAACGCGCAGTTGGACAACTACTTGCAGAACCGCCTGGCCGAA     480
M  E  H  H  E  D  N  A  Q  L  D  N  Y  L  Q  N  R  L  A  E       20

AGTCTGCAAATTTGCGGAGGCGCCGGCGAGCATAATCCTCATCTGGCAGACGCTACTGGG     540
S  L  Q  I  C  G  G  A  G  E  H  N  P  H  L  A  D  A  T  G       40

GGGAACGGCTGCGCTCCTGGCATTGCGCCCTCAAAGTCGGACGAAGTCGACGGGGAAGAA     600
G  N  G  C  A  P  G  I  A  P  S  K  S  D  E  V  D  G  E  E       60

GACGAGGAGTGGAAGTACATACACGAGGTGCGGCAGAGTGAAAAGCTTCAACAGGAAAAG     660
D  E  E  W  K  Y  I  H  E  V  R  Q  S  E  K  L  Q  Q  E  K       80

CTTCCACTGACAAAGGAAACCGGCAATGGCTTCGGGCCCGGACGGGACTCAGATAATCAA     720
L  P  L  T  K  E  T  G  N  G  F  G  P  G  R  D  S  D  N  Q      100

GTTCACGGAAACGGAGCTGCCGCTGTCTTCAATCTGTACGAAGAGGACGTGGAAGTGATC     780
V  H  G  N  G  A  A  A  V  F  N  L  Y  E  E  D  V  E  V  I      120

AAGAACGATGGTGACTTCTCGACAAATTCTAACACCACTACTTCAACGGACGAGGTAGTT     840
K  N  D  G  D  F  S  T  N  S  N  T  T  T  S  T  D  E  V  V      140

GCCAGGCAGGCCCAGGAGCCAAACCAGTTGCCGGAGCAGCTTCAACAGCAGCAGCAGATA     900
A  R  Q  A  Q  E  P  N  Q  L  P  E  Q  L  Q  Q  Q  Q  Q  I      160

GAATCACAAGGTGTTCACGAGGATCCCCGTCAGGAAGATGAGGATGAGCACAGCTCCGTG     960
E  S  Q  G  V  H  E  D  P  R  Q  E  D  E  D  E  H  S  S  V      180

GCCACTACTTATGGAACAAGCAGTCTCAGTGAGAACAACTCCTCGCCTTTAGATCAGGAG    1020
A  T  T  Y  G  T  S  S  L  S  E  N  N  S  S  P  L  D  Q  E      200

GAGGTCGTTATGGTGGCCCAAACGGTTGGTCAAGAGCAGTTAGTGGACTTCGACAACAAG    1080
E  V  V  M  V  A  Q  T  V  G  Q  E  Q  L  V  D  F  D  N  K      220

GAAAACTCCTATTTTGTTAAAAACCTGGAAGAAAACCACTCGCAGCTAAACCCCAATGCT    1140
E  N  S  Y  F  V  K  N  L  E  E  N  H  S  Q  L  N  P  N  A      240

GTGGCCTTTGTGCCTGGCGTTGGCTCACAATCATCCTCTCCCCTTCCTGCTGCTGAAGAT    1200
V  A  F  V  P  G  V  G  S  Q  S  S  S  P  L  P  A  A  E  D      260

CCACTACCTGGTGTTCAGCCCCGCCCATTTCTACCGGGAGGGACCCTTGACGATTTGGTG    1260
P  L  P  G  V  Q  P  R  P  F  L  P  G  G  T  L  D  D  L  V      280

GCGGAAAGTCCGCGGAAAGAATTCGCCCGAATCAACATGGATGGTATTGCCGTGCCCGAC    1320
A  E  S  P  R  K  E  F  A  R  I  N  M  D  G  I  A  V  P  D      300

GAGCGGGAATTTGACATTGAAGCGGATATGCGACCCCACGAGTTGGAGCAGGAATCGGAC    1380
E  R  E  F  D  I  E  A  D  M  R  P  H  E  L  E  Q  E  S  D      320

ACTTTTGGGGCAGGACACCTGGAGATGCAGTTGTTGAACGGTATAGGAACCGCTGACCAA    1440
T  F  G  A  G  H  L  E  M  Q  L  L  N  G  I  G  T  A  D  Q      340

GCAGCCTTAAGGGATGTTCTGGATCACGGTCCAGAGACAAGTGTCGATATGGAGTTGCCA    1500
A  A  L  R  D  V  L  D  H  G  P  E  T  S  V  D  M  E  L  P      360

CTAGATCAGGTGCCCAACGACGCCGACATCATGAAACAATCGATTTATGCCGAGCACAAC    1560
L  D  Q  V  P  N  D  A  D  I  M  K  Q  S  I  Y  A  E  H  N      380

TCCTCCATTGAAGATATCTTAAATTCCGTGCAACCTTTGCCAATTCAGACTTGTGACGAT    1620
S  S  I  E  D  I  L  N  S  V  Q  P  L  P  I  Q  T  C  D  D      400

AAGGAACTAATTCATGTGGAAGAGAAAGAACATGTCTCGAAGTCGCCTTCTACAGAGGAG    1680
K  E  L  I  H  V  E  E  K  E  H  V  S  K  S  P  S  T  E  E      420

CTCCAGTTTCAGTCCGACTTCCCAAACAATCAGGAATCGCACACATTATTCAATAACACT    1740
L  Q  F  Q  S  D  F  P  N  N  Q  E  S  H  T  L  F  N  N  T      440

GAGCAGGATCCAATGCAGGCTTCATTTTATTTGGAACATACCTCTCAAAAGGCTCAAGAG    1800
E  Q  D  P  M  Q  A  S  F  Y  L  E  H  T  S  Q  K  A  Q  E      460

GGCTGCCAGGAGCAAATGCAGCTTCCCGCTGAGTGCTCTGATATTTTTGCCGACCAGAGT    1860
G  C  Q  E  Q  M  Q  L  P  A  E  C  S  D  I  F  A  D  Q  S      480

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L  L  L  D  T  S  A  P  Q  L  S  S  E  A  D  S  P  V  A  K      500

TTGGAGCTGGAATCCCAGCAAGCTGGCATTGTAGACATCACACCTTCGCCCCTTTCTTCC    1980
L  E  L  E  S  Q  Q  A  G  I  V  D  I  T  P  S  P  L  S  S      520

ACTGCAGAGAAACATCTGGTTGAAGACACCAAGGAGCTAGTTGAGGAGTACACGTTGGAT    2040
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CCGGAATCCCATTTCTTTGGGGTTGTTTCATCTCAAGCTCCTCTACAGAACGACGAGGAA    2100
P  E  S  H  F  F  G  V  V  S  S  Q  A  P  L  Q  N  D  E  E      560

AATGCTGTTTTTGAATCAGTTTCTGGTTATGAAACTCAAAACTTTGATGAAATATCATCT    2160
N  A  V  F  E  S  V  S  G  Y  E  T  Q  N  F  D  E  I  S  S      580

CCACCGGAAGGTATTAATCCGTTTGCCCAGCCCTTCACGCCCGCCCACCTGGTGATAGAG    2220
P  P  E  G  I  N  P  F  A  Q  P  F  T  P  A  H  L  V  I  E      600

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Q  A  N  T  M  M  E  D  V  G  G  M  P  I  P  A  S  E  D  F      620

GCTATATGTGATAAAGTTGCTAGTAAGTCTTCAAATGAAGTAGAGGATCATAGGAGTGAG    2340
A  I  C  D  K  V  A  S  K  S  S  N  E  V  E  D  H  R  S  E      640

CAACAAGCCTTTGTAAAGGAGGAGCTGCTGCACCCTGTTGGAGATGTTGTTGCCCAGGTT    2400
Q  Q  A  F  V  K  E  E  L  L  H  P  V  G  D  V  V  A  Q  V      660

GAAAATCTTGGCACTGAAAAGAACTTCGTTGTTGAAGAAGAGCGACTTCCTATTTCTGTT    2460
E  N  L  G  T  E  K  N  F  V  V  E  E  E  R  L  P  I  S  V      680
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S  D  E  I  P  L  S  S  A  S  K  E  K  L  L  P  D  T  T  D      700

GAGCAACTGCTGACTTCTGCTTTGGAGGAGAAACTGCGCTCTGTTGCTCCAGAGGAGTCA    2580
E  Q  L  L  T  S  A  L  E  E  K  L  R  S  V  A  P  E  E  S      720

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V  S  T  A  A  D  G  Q  S  I  S  Q  F  D  E  Y  V  I  A  S      740

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N  K  P  L  E  D  I  L  E  P  E  K  D  V  E  V  A  K  S  L      760

TCTGAAAAAACTTCTGTTGCTACAGTGGCTGGAGGTGCTGTGGTTGGTGCTACCAAAACC    2760
S  E  K  T  S  V  A  T  V  A  G  G  A  V  V  G  A  T  K  T      780

CACTCAGCCACCAAAGCAGGATCTACAGCAGCTAGTGCAAAATCGAAAACAGAAACCCTT    2820
H  S  A  T  K  A  G  S  T  A  A  S  A  K  S  K  T  E  T  L      800

GTAATGAAGAAGACTACAGCGAGTTCTACTTCTGTTTACGGGGCAAACAAGTCGGCCGCT    2880
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AAGCCTAAAGTTTTGTCGCCGCGCAGCACCATTTCCTCCACCACCACTGTGCGAAAGGTG    3360
K  P  K  V  L  S  P  R  S  T  I  S  S  T  T  T  V  R  K  V      980

CCTAGCACAAGTACCCCTTCATTTTCTACTCGTAGCCCTAACAAGCAGCAATCCAATGGA    3420
P  S  T  S  T  P  S  F  S  T  R  S  P  N  K  Q  Q  S  N  G     1000
                            **********

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L  G  K  N  T  S  S  T  T  S  T  A  T  A  T  I  T  K  S     1020

TTCACAGCTCGCTCAGCTCCAAAGTTTACGCATTCAGCTAGCTTAACCTATAACAATGGG    3540
F  T  A  R  S  A  P  K  F  T  H  S  A  S  L  T  Y  N  N  G     1040

AGTACATCTCGTCGTTTGCTGGTTCCTGGAAGCTCCTCTACTACAACTACGTCCTCTCTT    3600
S  T  S  R  R  L  L  V  P  G  S  S  S  T  T  T  T  S  S  L     1060

AGGAAGTCATCACCATTAAAGGCAGCACCAGGCAAAGCTGCTTCCAAGCCATTGACACCG    3660
R  K  S  S  P  L  K  A  A  P  G  K  A  A  S  K  P  L  T  P     1080

CAATCCAAAGATGGCACGGCTAAGTCATCGCCGGCTGTATTAAAAGCTCGGAATTCTACT    3720
Q  S  K  D  G  T  A  K  S  S  P  A  V  L  K  A  R  N  S  T     1100

TTGATGGGCGGGGAAGGGGTAGCACCATCAAATGAATGTGTCCCAACACCTAATGGTCAG    3780
L  M  G  G  E  G  V  A  P  S  N  E  C  V  P  T  P  N  G  Q     1120

ATCAATGCGGAAGAAGTGGTCAAGCTTAAGGGAAAGGGCCTAGCAGAAGAGGTTAAGATT    3840
I  N  A  E  E  V  V  K  L  K  G  K  G  L  A  E  E  V  K  I     1140

ATTGAAGAGCAAAAGCTTCACGAACAAGATGTCCCTGCCCATAATGCTGAAGTGCCCCTT    3900
I  E  E  Q  K  L  H  E  Q  D  V  P  A  H  N  A  E  V  P  L     1160

TTGGATTTTTAATATGTATCCTAAATTCCAGATCACACAAACCTAAAATTACTTTATTAA    3960
L  D  F  *

TGAAAACATATTGAGAAAAAGTGTTTAAAACGAACAGAAAAAAATTTCGAAGACGTAATA    4020

AGAAAAACGATGAATACAATAGCATCCTGGTAACCAAATTAGGAATTTTTGCTTATTGAA    4080

CTTATTTATATACGATTATACCCTCGATTTCTTGCTACATATTATTAAATCATTTTATTT    4140

CAAAAAATAATGAACTACTGTAATGAACGCAGAGCGAATAAATGTTTGTGCTATTTTGTA    4200
                                  ------

ACGAAAACGAAAGATAAATGTCACCAAGTGTTCTGTTATTTAAAAATTAAATATTTACTT    4260

TTGAAATGCTTTAAAAAAAATTTTTGCTCACGTTCTTAAATTCTTAAATTAAATTTCGTA    4320

CAACTTTTAAACTTTTAACAGCAGAAAACTTCGAAAACGTTAAAAGAAAAATGAAGAATA    4380

CAGTAGCATCCTCTTGCTTACCAAATTAGGATTTTCTGCTCATTGAACTTATTTATATAA    4440

TCTTTTACTTTATACCATAGTGAACTAATGTTTGTGCTACTTTGTAACCAAATCGAAAGA    4500

TCAATGTCACCATGTTCTCCGTTATTTAAAAATATAACAATTACATTTAAAATGTTTTTA    4560

ATACATTTTTTGATCACATTCGAAATTTCATTAAATTGCGTCAAACATTTACAAATTGTC    4620

CTTTATCAAAAAAAAAAAA
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Figure 3. DNA sequence of cDNA clones. (*A*) The complete sequence of cDNA B3 is shown. The deduced amino acid sequence of the longest ORF is indicated with capital letters below the DNA sequence. Numbers at the right margin indicate DNA positions and amino acid residues respectively. The protein sequence that represents the phosphorylation consensus sequence for the *cdc2* kinase is marked

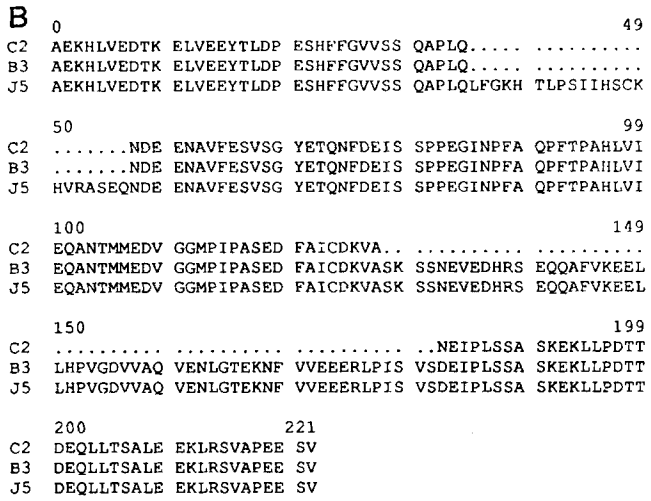

Figure 3.

that the region from amino acid 1 to 1453 is predominantly acidic, with a pI of 4.3, whereas the carboxy terminus from 1454 to 1828 is predominantly basic, with a pI of 11.4 (Fig. 4). The tau sequence is also composed of an acidic amino-terminal domain of 46 amino acids (pI = 3.7), and a large basic region (pI = 10.8) of 317 residues in the middle and a short acidic carboxy-terminal region. The comparison of the charge distribution of the 205K MAP and MAP2 shows that both proteins are similarly organized with regard to basic and acidic domains (Fig. 4).

The unusual distribution of charge in the 205K MAP sequence might also be the cause of the discrepancy between the predicted size of 130 kD and the mobility corresponding to 205 kD, as observed on SDS-polyacrylamide gels. In support of this suggestion, the migration behavior of a truncated protein, N723 (see next section), which lacks most of the acidic region, is not anomalous. The predicted size of this protein is 55 kD, which is consistent with the molecular mass of 63 kD estimated from SDS-polyacrylamide gels (Fig. 5). On the other hand, proteins that retain the acidic region all have anomalous mobilities.

One way to identify functionally important sequences is by virtue of homology to other well-understood proteins. We compared the amino acid sequence of the 205K MAP with known protein sequences using the UWGCG program Wordsearch (Devereux et al., 1984) and did not detect significant similarities. We also directly compared the 205K MAP sequence with the protein sequences of MAP2 (Lewis et al., 1988) and tau (Lee et al., 1988) by using the programs Bestfit and Compare (Devereux et al., 1984), and again we did not observe significant similarities. We conclude from these observations that the *Drosophila* 205K MAP is not related to other known MAPs by sequence homology. It does, however, consist of amino acids that give rise to a hydrophilic protein comparable to other MAPs mentioned above, and it is similar to these in the structural organization of basic and acidic protein domains.

205K MAP Isoforms May Result from Differential Splicing and Phosphorylation

Since the 205K MAP exists as multiple isoforms on SDS-polyacrylamide gels (Goldstein et al., 1986) we were interested in what might cause the variety of species. Some 205K MAP species might be the product of differentially spliced mRNAs; but an additional complexity of protein isoforms might be generated by posttranslational modifications such as phosphorylation.

We investigated the first possibility by comparing the content of coding sequences of the different cDNA clones that we had isolated. Our restriction analyses identified three forms of 205K MAP cDNA, presumably corresponding to three different mRNA forms. These three forms vary internally in the sequence and would encode different protein products. To determine if this were the case, we sequenced the Pst I–Hinc II fragment, which contains the variable region from representative types of these cDNAs. This analysis revealed the presence of three different coding sequences, which are summarized in Fig. 3 *B*. Interestingly, the size differences that each of these three imparts to the 205K MAP protein are small, but possibly on the order of the different protein species previously reported (Goldstein et al., 1986). The variant regions are upstream from, but not within the one functional region of the protein we know of, the microtubule-binding region. Whether these alternative regions confer novel regulatory or structural properties upon the 205K MAP remains to be determined. Searches of the protein database with the alternative exons revealed no proteins with substantial similarity.

Further examination of the predicted protein sequence revealed numerous sequences that match known consensus target sequences for several different protein kinases. For example, the protein sequence of the 205K MAP contains a sequence Arg-Ser-Pro-Gln-Lys at amino acid position 990 (Fig. 3), which fits the phosphorylation consensus sequence for *cdc2* or histone H1 kinase (Shenoy et al., 1989). In addition to the *cdc2* phosphorylation consensus sequence, a site at position 1060 (Arg-Lys-Ser-Ser) resembles sequences phosphorylated by cAMP-dependent kinase (PKA) (Edelmann et al., 1987). The sequence Ser-X-Lys/Arg-Lys/Arg, which is the consensus target for phosphorylation by calcium-phospholipid-dependent kinase (PKC) (Gonzales et al., 1989) occurs five times in the 205K MAP sequence at position 283, 927, 947, 1024, and 1040. The consensus for phosphorylation by a calcium–calmodulin-dependent kinase (PKII), Lys/Arg-X-X-Ser/Thr (Pearson et al., 1985), can be found 22 times in the 205K MAP sequence. Except for the phosphorylation site at position 414, all sites reside within the basic, carboxy-terminal portion of the protein. Evidence that some 205K MAP species are actually phosphorylated comes from in vivo labeling experiments of cultured *Dro-*

by asterisks. The DNA sequence representing a polyadenylation signal is underlined. These sequence data are available from EMBL/GenBank/DDBJ under accession number X54061. (*B*) The amino acid sequence of three differentially spliced cDNA clones in the region between restriction sites Pst I and Hinc II is presented. Amino acid position 1 is equivalent to position 522 in the protein sequence in *A* and position 221 corresponds to 721 in *A*.

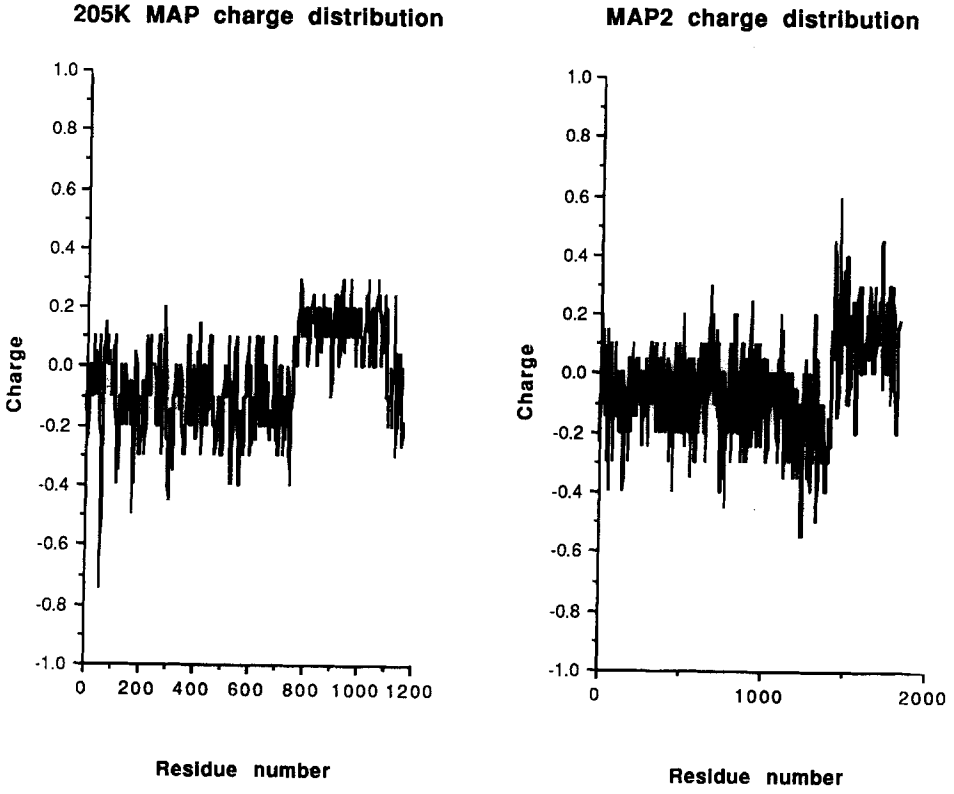

Figure 4. Charge distribution of *Drosophila* 205K MAP and mouse MAP2. The average charge per residue was calculated for a sliding window of 10 amino acids and plotted for both 205K MAP and MAP2.

sophila cells followed by immunoprecipitation with 205K MAP antibodies (Pesavento, P., and L. Goldstein, unpublished).

Determination of the Microtubule-binding Region of the 205K MAP

To begin elucidation of the mechanism whereby the 205K MAP might play a role in the regulation of microtubule assembly or interaction, we mapped the region responsible for microtubule binding. We conducted this analysis by synthesizing proteins in vitro from clones lacking various regions of coding sequence. The resulting proteins were tested for their ability to bind to polymerized microtubules. Representative data from these experiments are shown in Fig. 5. Under our conditions, the larger clones and proteins experienced either internal initiation, incomplete translation, or degradation in vitro, leading to variable arrays of proteins smaller than the longest species that should be synthesized by each clone. For the microtubule binding experiments, we followed the behavior of the largest species only, owing to uncertainty as to whether the smaller proteins resulted from alteration at the amino or carboxy ends.

A map of the internally deleted and truncated proteins that we studied is shown in Fig. 6. The carboxy-terminally truncated proteins (C1023, C955, et cetera) were expressed from the truncated cDNA clones used for sequencing. The numbers in their names indicate their predicted length in amino acids. The internally deleted proteins, N386 and N723, were expressed from internally deleted clones, which were created by ligating the Sal I restriction site (nucleotide position 585) to either the Eco RV site (nucleotide position 1573) or to the Hinc II site (nucleotide position 2583). The resulting proteins contained 55 amino acids of the amino terminus fused inframe to amino acid 386 (N386) or amino acid 723 (N723).

The region sufficient to bind to microtubules could be localized within the carboxy-terminal third of the molecule (Fig. 6). The carboxy-terminally truncated protein C1023, which lacks amino acids after residue 1023, and a shorter truncated protein, C955, which lacks amino acids after residue 955, both bind to microtubules as well as the full-size protein (Figs. 5 and 6). All carboxy-terminally truncated proteins shorter than C955 bind partially (C928, C920, and C813) or not at all (C774, et cetera). For example, in the case of the shortest of the partially binding truncated proteins, C813, ~70% of the protein fails to bind to microtubules, and remains in the supernatant in the binding assays. The proteins N386 and N723, in which internal regions are deleted, both bound microtubules fully.

Based on the behavior of the smallest proteins that retained full activity (N723 and C955), we conclude that the maximal region necessary for full microtubule-binding activity consists of 232 amino acids ranging from amino acid position 723 to 955, within the basic domain of the protein. There are two major interpretations of the partial binding activity of proteins C928, C920, and C813. One possibility is that the actual binding region is smaller than the 232-amino acid region defined by the proteins with full binding activity. In this case the partial binding activity of C928, C920, and C813 may result from deletion of elements necessary for proper folding of the microtubule-binding element. Alternatively, there may be multiple elements needed for microtubule-binding within the 232-amino acid region. In this case, the partial binding activity of C928, C920, and C813 may reflect deletion of some, but not all, of these redundant elements. We note that if there are redundant binding elements, then

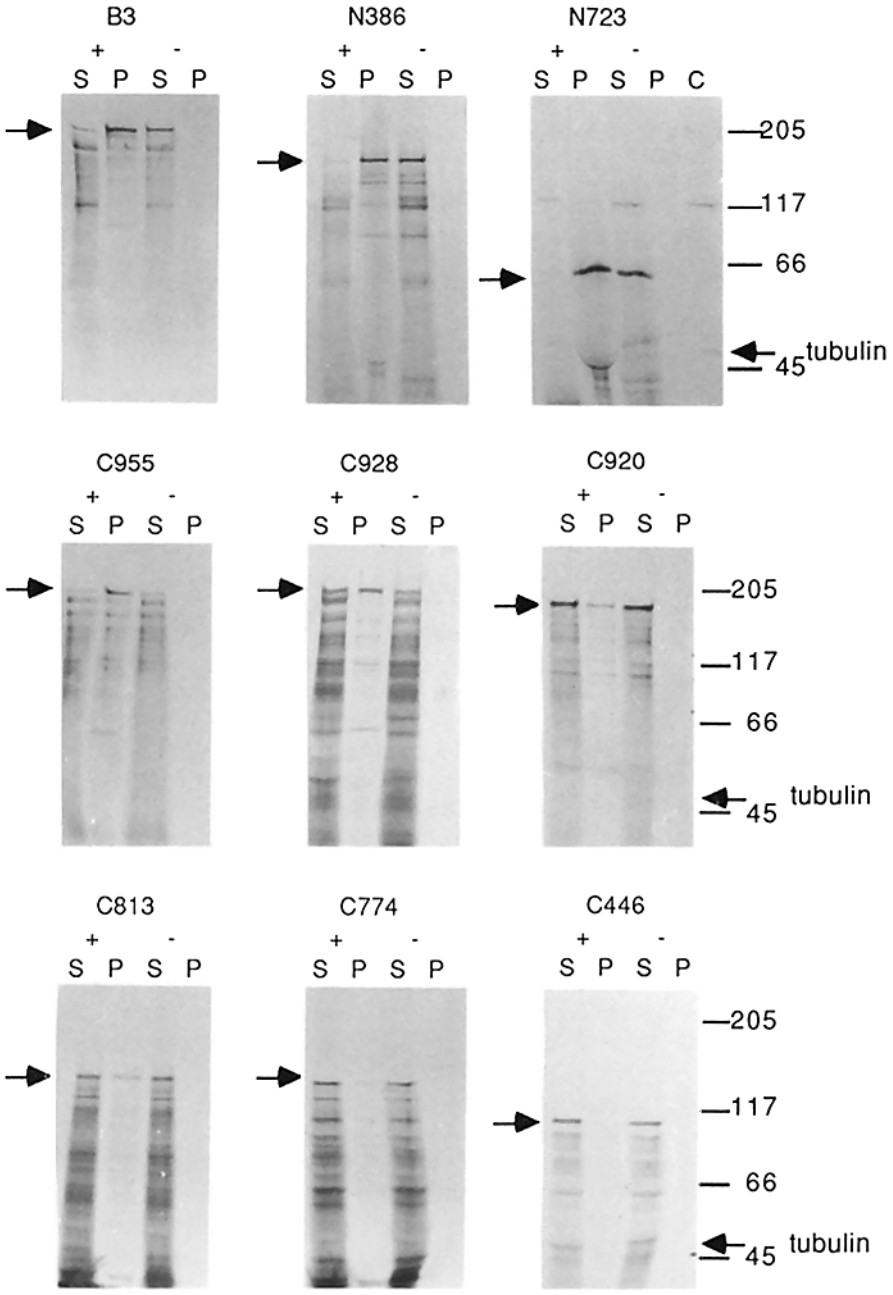

Figure 5. Microtubule binding assay with in vitro generated full-length and truncated 205K MAP proteins. In vitro generated truncated proteins and full-length 205K MAP (from cDNA B3) were tested for microtubule binding. Supernatant and pellet fractions, loaded onto SDS-polyacrylamide gels are indicated with S and P respectively. + and −, sedimentation in the presence or absence of microtubules. (*Top, right*) Lane *C* shows the endogenous translation products of a reticulocyte lysate mix incubated without 205K MAP mRNA. Arrows indicate the largest protein species whose binding behavior is followed. The approximate position of the tubulin is shown in each row.

they do not have similar sequences since we find no repeated sequence elements within this 232 amino acid region.

Discussion

In this paper, we report the predicted amino acid sequence of the *Drosophila* 205K MAP. The amino acid sequence suggests that the 205K MAP is a hydrophilic protein. The distribution of charged residues predicts a polarized structure formed by distinct acidic and basic regions of the protein. The region that is essential for microtubule binding is located in the basic carboxy-subterminal part of the protein (see Fig. 7).

One important finding about 205K MAP gene structure was the identification of embryonic cDNAs that represent differentially spliced mRNAs. At this time, we do not know whether some of the different species are specifically expressed at particular stages of development. However, complex patterns of transcription and translation have been reported for many genes. Interestingly one particular MAP, tau, is known to be differentially spliced and for two forms of tau protein it could be shown that their expression is developmentally regulated (Goedert et al., 1989). It will be interesting to see whether different forms of the 205K MAP possess different functions.

To understand the functions of the 205K MAP, it is essential to know its overall structure. Currently, in the MAP4 family, such information is only available for the HeLa 210K MAP, which is immunologically related to the 205K MAP (West, R. R., K. M. Tenbarge, M. Gorman, L. S. B. Gold-

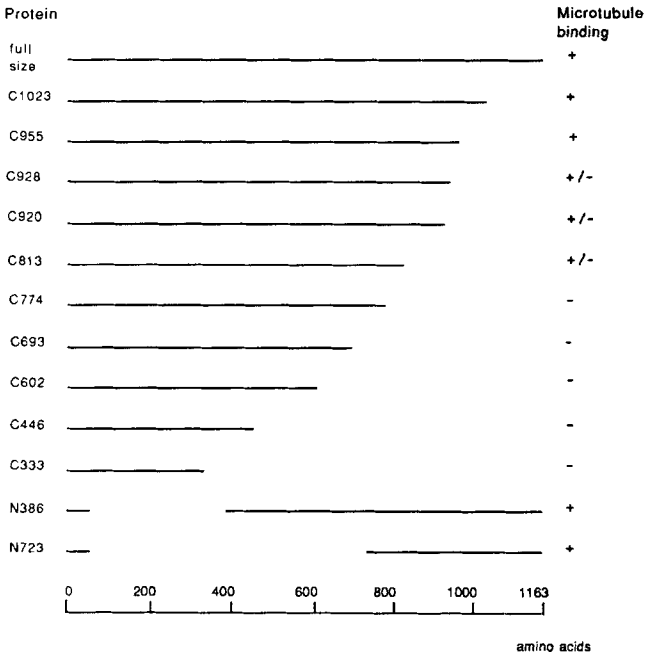

Figure 6. The size and binding ability of full-sized and truncated proteins is indicated. Lines indicate the regions of the protein that are present.

stein, and J. M. Olmsted. 1988. *J. Cell Biol.* 107:460a) and is by several criteria a member of the MAP4 family. The HeLa 210K MAP has been reported to possess a highly asymmetric structure (Bulinski and Borisy, 1980). MAP2 and tau have also been reported to exist as rodlike structures on microtubules as observed by EM (Kim et al., 1986; Vallee, 1980; Voter and Erickson, 1982; Hirokawa et al., 1988). In the case of tau, biochemical data also indicate that it is a highly asymmetric molecule with little helical content (Cleveland et al., 1977*b*). Because of the similar arrangement of different protein domains in MAP2 and 205K MAP, and the similarities to MAP4, we suggest that an elongate structure is likely for the 205K MAP.

The location of the binding domain of *Drosophila* 205K MAP within the protein is similar to that of MAP2 and tau, in which this region is within the positively charged domain (Lewis et al., 1988; Himmler et al., 1989). There is no sequence similarity at the amino acid level between the microtubule-binding region of the *Drosophila* 205K MAP, and the repeated amino acid sequences implicated in microtubule binding by MAP2 or tau. In addition, there is no sequence similarity to the microtubule-binding domain of *Drosophila* kinesin (Yang et al., 1989), mammalian MAP1B (Noble et al., 1989) or the peptide repeats of the *Trypanosoma* 320K MAP (Schneider et al., 1988). In this context, we note that unlike the *Drosophila* 205K MAP, the bovine adrenal 190-kD MAP, which is also thought to be a member of the MAP4 family, contains a 17-amino acid sequence which is similar to the peptide repeats found in the binding domains of MAP2 and tau (Aizawa et al., 1989). Why the *Drosophila* 205K MAP has a different microtubule-binding region than the bovine adrenal 190K MAP is unclear at present. Thus, the *Drosophila* 205K MAP adds a fifth binding domain motif to the class of microtubule-binding proteins. The finding that tau, MAP2, and the 205K MAP, all have a microtubule-binding region that is within a very basic region of the protein is consistent with previous findings that the MAP-binding position of tubulin is acidic and that the MAP–microtubule interaction is electrostatic (Maccioni et al., 1988; Paschal et al., 1989).

The finding of numerous potential kinase target sequences in the 205K MAP raises the possibility of its being regulated by phosphorylation. Other investigators have found that MAPs can be purified with varying amounts of phosphates incorporated (Hagested et al., 1989; Tsuyama et al., 1987). Furthermore, phosphorylation of MAP2 and tau by Ca^{2+} calmodulin-dependent protein kinase and cAMP-dependent protein kinase in vitro has been shown to inhibit MAP-induced microtubule polymerization (Burns et al., 1984; Goldenring et al., 1985; Yamamoto et al., 1984). Most recently it has been reported that microtubule dynamics during the interphase–metaphase transition can be correlated with the activity of the *cdc2* protein kinase in extracts of *Xenopus* eggs (Verde et al., 1990). Consistent with this finding, the microinjection of *cdc2* into mammalian fibroblasts (Lamb et al., 1990) leads to numerous structural changes, including changes in microtubule organization. If the role of the 205K

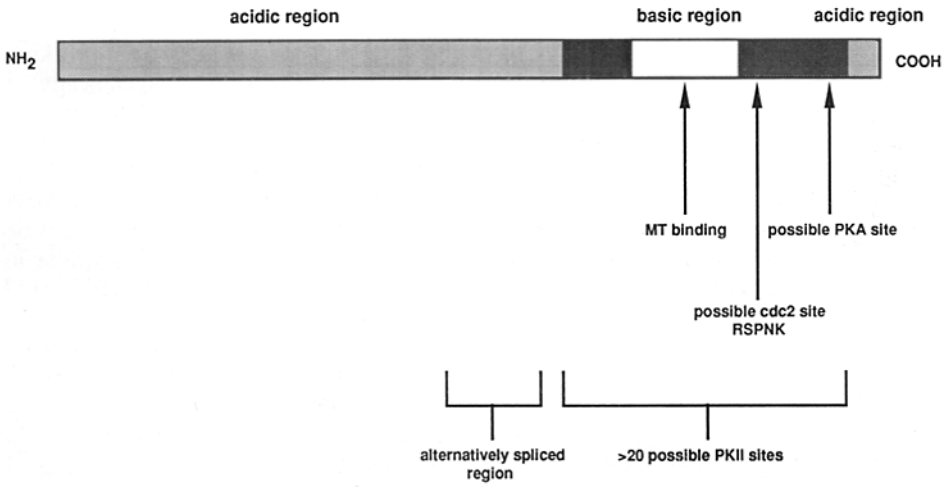

Figure 7. Summary of 205K MAP organization. The acidic and basic regions of 205K MAP are indicated as well as the position of the putative microtubule binding site. The location of the consensus sequences for protein kinases are marked with arrows and the consensus sequence for the cdc2 kinase is given. Abbreviation for protein kinases are: cyclic AMP-dependent protein kinase (*PKA*) and calcium–calmodulin-dependent kinase (*PKII*).

MAP in MT assembly is regulated by phosphorylation, a cell cycle–regulatory kinase such as *cdc2* (MPF) could lead to regulation of MAP binding to microtubules during mitosis.

To conclude, MAP4 type proteins have been identified in many species (Bulinski and Borisy, 1979; Olmsted, 1986; Murofushi et al., 1986; Kotani et al., 1988) as 200-kD proteins colocalized with microtubules of the mitotic spindle and cytoplasm (Bulinski and Borisy, 1980; Kuriyama et al., 1984; Parysek et al., 1984; Goldstein et al., 1986). The function of the MAP4 class proteins might be distinct from that of MAP2 and tau proteins, which are localized primarily in neurons (Bernhardt and Matus, 1984; Binder et al., 1985). Surprisingly, the overall asymmetric structure of 205K MAP is similar to the structural organization of MAP2 without sharing precise sequence similarity. Whether this structural similarity reflects a functional similarity remains to be determined. Finally, our data have suggested that the functions of the 205K MAP may be regulated at two levels: (*a*) by alternative splicing to generate different proteins and (*b*) by phosphorylation.

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References

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

Bernhardt, R., and A. Matus. 1984. Light and electron microscopic studies of the distribution of microtubule-associated protein 2 in rat brain: a difference between dendritic and axonal cytoskeletons. *J. Comp. Neurol.* 226:203–231.

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

Bloom, G. S., F. Luca, and R. B. Vallee. 1984. Widespread cellular distribution of MAP1A (microtubule-associated protein 1A) in the mitotic spindle and on interphase microtubules. *J. Cell Biol.* 98:331–340.

Brown, N. H., D. L. King, M. Wilcox, and F. C. Kafatos. 1989. Developmentally regulated alternative splicing for *Drosophila* integrin PS2 transcripts. *Cell.* 59:185–195.

Bulinski, J. C., and G. G. Borisy. 1979. Self-assembly of microtubules in extracts of cultured HeLa cells and the identification of HeLa microtubule-associated protein. *Proc. Natl. Acad. Sci. USA.* 76:293–297.

Bulinski, J. C., and G. G. Borisy. 1980. Microtubule-associated proteins from cultured HeLa cells. *J. Biol. Chem.* 255:11570–11576.

Burns, R., K. Islam, and R. Chapman. 1984. The multiple phosphorylation of the microtubule-associated protein MAP2 controls the MAP2:tubulin interaction. *Eur. J. Biochem.* 141:609–615.

Cavener, D. R. 1987. Comparison of the consensus sequence flanking translational start sites in Drosophila and vertebrates. *Nucleic Acids Res.* 15:1353–1361.

Cleveland, D. W., S.-Y. Hwo, and M. W. Kirschner. 1977*a*. Purification of tau, a microtubule-associated protein that induces assembly of microtubules from purified tubulin. *J. Mol. Biol.* 116:207–225.

Cleveland, D. W., S.-Y. Hwo, and M. W. Kirschner. 1977*b*. Physical and chemical properties of purified Tau factor and the role of Tau in microtubule assembly. *J. Mol. Biol.* 116:227–247.

Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.

Drubin, D. S. K., and M. Kirschner. 1986. Association of tau protein with microtubules in living cells. *Ann. NY Acad. Sci.* 466:257–268.

Edelmann, A. M., D. K. Blumenthal, and E. G. Krebs. 1987. Protein serine/threonine kinases. *Annu. Rev. Biochem.* 56:567–613.

Goedert, M., C. M. Wischik, R. A. Crowther, J. E. Walker, and A. Klug. 1988. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimers disease: identification as the microtubule-associated protein tau. *Proc. Natl. Acad. Sci. USA.* 85:4051–4055.

Goedert, M., M. G. Spillantini, M. C. Potir, J. Ulrich, and R. A. Crowther. 1989. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:393–399.

Goldenring, J. R., M. L. Vallano, and R. J. DeLorenzo. 1985. Phosphorylation of microtubule-associated protein 2 at distinct sites by calmodulin-dependent and cyclic-AMP-dependent kinases. *J. Neurochem.* 45:900–905.

Goldstein, L. S. B., R. A. Laymon, and J. R. McIntosh. 1986. A microtubule-associated protein in *Drosophila melanogaster*: Identification, characterization and isolation of coding sequences. *J. Cell Biol.* 102:2076–2087.

Gonzales, G. A., K. K. Yamamoto, W. H. Fischer, D. Karr, P. Menzel, W. Biggs III, W. W. Vale, and M. R. Montminy. 1989. A cluster of phosphorylation sites on the cyclic AMP-regulated nuclear factor CREB predicted by its sequence. *Nature (Lond.).* 337:749–752.

Hagested, T. M., B. Lichtenberg, H. Wille, E. M. Mandelkow, and E. Mandelkow. 1989. Tau becomes long and stiff upon phosphorylation: Correlation between paracrystalline structure and degree of phosphorylation. *J. Cell Biol.* 109:1643–1651.

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

Hirokawa, N., Y. Shiomura, and S. Okabe. 1988. Tau proteins: the molecular structure and mode of binding on microtubules. *J. Cell Biol.* 107:1449–1459.

Izant, J. G. 1981. Immunological and biochemical investigations of microtubule-associated proteins in the mitotic spindle and interphase cytoplasm of mammalian cells. Ph.D. thesis. University of Colorado, Boulder, CO.

Izant, J., J. Weatherbee, and J. R. McIntosh. 1983. A microtubule-associated protein antigen unique to mitotic spindle microtubules in PtK1 cells. *J. Cell Biol.* 96:424–434.

Kim, H., C. Jensen, and L. I. Rebhun. 1986. The binding of MAP2 and tau on brain microtubules in vitro; implications for microtubule structure. Dynamic aspects of microtubule biology. *Ann. NY Acad. Sci.* 466:218–239.

Kotani, S., H. Murofushi, S. Maekawa, H. Aizawa, K. Kaji, and H. Sakai. 1987. Identification of the 190kD microtubule-associated protein in cultured fibroblasts and its association with interphase and mitotic microtubules. *Cell Struct. Funct.* 12:1–9.

Kotani, S., H. Murofushi, S. Maekawa, H. Aizawa, and H. Sakai. 1988. Isolation of rat liver microtubule-associated proteins. *J. Biol. Chem.* 263:5385–5389.

Kuriyama, R., G. Keryer, and G. G. Borisy. 1984. The mitotic spindle of chinese hamster ovary cells isolated in taxol-containing medium. *J. Cell Sci.* 66:265–275.

Lamb, N. J. C., A. Fernandex, A. Watrin, J. C. Labbé, and J. C. Cavadore. 1990. Microinjection of $p34^{cdc2}$ kinase induces marked changes in cell shape, cytoskeletal organization and chromatin structure in mammalian fibroblasts. *Cell.* 60:151–165.

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

Lewis, S. A., D. Wang, and N. J. Cowan. 1988. Microtubule-associated protein MAP2 shares a microtubule-binding motif with tau protein. *Science (Wash. DC).* 242:936–939.

Maccioni, R. B., C. I. Rivas, and J. C. Vera. 1988. Differential interaction of synthetic peptides from the carboxyl-terminal regulatory domain of tubulin with microtubule-associated proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1957–1963.

Maniatis, T., R. C. Hardison, E. Lacy, J. Lauer, C. O'Connell, and D. Quon. 1978. The isolation of structural genes from libraries of eucariotic DNA. *Cell.* 15:687–701.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1983. Molecular Cloning: A Laboratory Manual. Cold Spring Laboratory, Cold Spring Harbor, NY. 545 pp.

Murofushi, H., S. Kotani, A. Aizawa, H. Hisanaga, N. Hirokawa, and H. Sakai. 1986. Purification and characterization of a 190kD microtubule-associated protein from bovine adrenal cortex. *J. Cell Biol.* 103:1911–1919.

Murphy, D. B., R. B. Vallee, and G. G. Borisy. 1977. Identity and polymerization-stimulatory activity of the nontubulin proteins associated with microtubules. *Biochemistry.* 16:2598–2605.

Noble, M., S. A. Lewis, and N. J. Cowan. 1989. The microtubule binding domain of Microtubule-associated Protein MAP1B contains a repeated sequence motif unrelated to that of MAP2 and tau. *J. Cell Biol.* 109:3367–3376.

O'Connell, P., and M. Roshash. 1984. Sequence, structure and codon preference of the *Drosophila* ribosomal protein 49 gene. *Nucleic Acids Res.* 12:5495–5513.

Olmsted, J. B. 1986. Microtubule-associated proteins. *Annu. Rev. Cell Biol.* 2:419–455.

Olmsted, J. B., and H. D. Lyon. 1981. A microtubule-associated protein specific to differentiated neuroblastoma cells. *J. Biol. Chem.* 256:3507–3511.

Parysek, L. M., C. F. Asnes, and J. B. Olmsted. 1984*a*. MAP 4: occurrence in mouse tissue. *J. Cell Biol.* 99:1309–1315.

Parysek, L. M., J. J. Wolosewick, and J. B. Olmsted. 1984*b*. A microtubule-associated protein specific for a subset of tissue microtubules. *J. Cell Biol.* 99:2287–2296.

Paschal, B. M., R. A. Obar, and R. B. Vallee. 1989. Interaction of brain cytoplasmic dynein and MAP2 with a common sequence at the C terminus of tubulin. *Nature (Lond.).* 342:569–572.

Pearson, R. B., J. R. Woodgett, P. Cohen, and B. E. Kemp. 1985. Substrate specificity of a multifunctional Calmodulin-dependent protein kinase. *J. Biol. Chem.* 260:14471–14476.

Sanger, F., S. Miklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

Sato-Yoshitake, R., Y. Shiomura, M. Miyasaka, and N. Hirokawa. 1989. Microtubule-associated protein 1B: Molecular structure, localization, and phosphorylation dependent expression in developing neurons. *Neuron.* 3:229–238.

Schneider, A., A. Hemphill, T. Wyler, and T. Seebeck. 1988. Large microtubule-associated protein of T. brucei has tandemly repeated, near-identical sequences. *Science (Wash. DC).* 241:459–462.

Shenoy, S., J.-K. Choi, S. Bagrodia, T. Copeland, J. Maller, and D. Shalloway. 1989. Purified maturation promoting factor phosphorylates pp60 c-src at the sites phosphorylated during fibroblast mitosis. *Cell.* 57:763–774.

St. Johnston, D. R., M. F. Hoffmann, R. K. Blackman, D. Segal, R. Grimaila, R. W. Padgett, H. A. Irick, and W. M. Gelbart. 1990. The molecular organization of the *decapentaplegic* gene in *Drosophila melanogaster*. *Genes & Dev.* In press.

Tsuyama, S., Y. Terayama, and S. Matsuyama. 1987. Numerous phosphates of microtubule-associated protein 2 in living rat brain. *J. Biol. Chem.* 262: 10886–10892.

Vallee, R. B. 1990. Structure and phosphorylation of microtubule-associated protein 2 (MAP2). *Proc. Natl. Acad. Sci. USA.* 77:3206–3210.

Vallee, R. B. 1982. A taxol-dependent procedure for the isolation of microtubules and microtubule-associated proteins (MAPs). *J. Cell Biol.* 92:435–442.

Verde, F., J. C. Labbé, M. Dorée, and E. Karsenti. 1990. Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of Xenopus eggs. *Nature (Lond.).* 343:233–238.

Voter, W. A., and H. P. Erickson. 1982. Electron microscopy of MAP2 (microtubule associated protein 2). *J. Ultrastruct. Res.* 80:374–382.

Wickens, M. S., and P. Stephenson. 1984. Role of the conserved AAUAAA sequence: four AAUAAA point mutants prevent messenger RNA 3′ end formation. *Science (Wash. DC).* 226:1045–1051.

Yamamoto, H., K. Fukunaga, S. Goto, E. Tanaka, and E. Miyamoto. 1985. Ca^{2+}, Calmodulin-dependent regulation of microtubule formation via phosphorylation of microtubule-associated protein 2, tau factor, and tubulin, and comparison with the cyclic AMP-dependent phosphorylation. *J. Neurochem.* 44:759–767.

Yang, J. T., R. A. Laymon, and L. S. B. Goldstein. 1989. A three domain structure of the kinesin heavy chain revealed by DNA sequence and microtubule-binding analysis. *Cell.* 56:879–889.